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HYBRID VIGOR AND WEIGHT OF GERMS IN THE SEEDS OF MAIZE [1]

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INTRODUCTION

The stimulation of growth resulting from hybridization remains one of the unexplained biological facts. The fact itself has been recognized from very early times and is the basis of the current effective system for improvement in maize and other crop plants. But just what takes place in the union of unlike germ plasms that brings about the observed stimulation has not been determined. The exact nature of the reaction between diverse hereditary elements is of great theoretical as well as practical interest, and until this problem has been solved all facets of hybrid vigor will continue to merit attention.

PREVIOUS INVESTIGATIONS

The demonstration of hybrid vigor in resting embryos, as distinct from seed size, was made by Ashby (*1*, *2*).[2] Using maize as his test plant, he investigated the possibility that the increased size of the mature hybrid organism over that of its parents was determined by the weight of the embryo and not by an increased rate of growth of the developing hybrid plant over that of the parents. Ashby concluded that hybrid corn plants grew at the same rate as their more rapidly growing parent and that their larger mature size followed from the fact that they had a larger embryo to start with, i. e., more capital invested. He subsequently extended his studies to other species and reached the same conclusions (*3*).

Ashby's conclusions as to the nature of hybrid vigor have been criticized by East (*7*) and amplified by Ashby (*4*, *5*). There can be no difference of opinion as to the facts. Hybrid embryos are often heavier than those of their parents. The divergence of opinion comes with the question as to whether all the increase in mature size of a hybrid over its parents can be attributed to the initial advantage of a larger embryo and, therefore, to a higher rate of growth, limited to the period before dormancy.

Obviously, if hybrid embryos produced on inbred plants are heavier than inbred embryos on the same or sister plants, they must grow either for a longer period or at a more rapid rate if growth is under-

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[2] Italic numbers in parentheses refer to Literature Cited, p. 80.

stood to mean an increase in dry weight, whether resulting from larger cells or more of them.

Lindstrom (*8*), using mutilated maize plants and failing to grasp Ashby's main thesis, believed he had demonstrated that the larger size of hybrid plants was not the result of a greater initial capital.

Sprague (*12*) showed, with some of the same seed stocks as Ashby, that reciprocal crosses differing in germ size attained identical final weights. He also pointed out the fallacy of Lindstrom's reasoning and reported a cross where the embryos were no heavier than those of one of the parent inbreds, yet the final weight of the hybrid plant was twice that of the inbred plant. Sprague concluded that the rate of growth of hybrids in the earlier stages of development was more rapid than that of the inbreds and that the increased final size was compounded of a more rapid growth rate and a larger initial embryo size. He believed his experiments were more readily explained on the basis of dominant favorable genes than on the more subtle physiologic stimulation resulting from the union of unlike germ plasms.

Paddick and Sprague (*10*), experimenting with maize, measured the relation of increase in hybrid germ weight, over that of the female parent, to the resulting forage and grain weights. Twenty-four hybrids were tested in 1 year and 22 in another. There was a low correlation of germ-weight increase with grain yield both years ($r=0.42$ and $r=0.36$) but no correlation with yield of stover.

These authors also measured the variation of kernel parts of an inbred line of maize and of several hybrids with it. They pointed out that, irrespective of seed size (which varies with position on the ear and with years), the ratio of germ to endosperm remains relatively constant.

Evidence was also presented of very great differences between reciprocal crosses in weight of seed parts. Of the nine reciprocal crosses tested, there were five hybrids that had germs lighter in weight than those of the female parent, and four of these were below both parents in germ weight. These hybrids, therefore, although presumably giving the customary mature-plant increases in weight, showed no evidence of heterosis in their germs.

Passmore (*11*), working with *Cucurbita pepo*, where seed size and embryo size correspond and there are no complications with endosperm, showed that where reciprocal F_1 hybrids differed by two to three times in germ weight the resulting plants were not greatly different at maturity. However, the heavier seeds gave a decided initial advantage, which became less with time. The final differences were equalized partially, because the plants from the small embryos matured later. Passmore made no direct statement as to the effect of heterosis on embryo size but left the inference that there was none. She stated, "Since the size of the F_1 seed is determined by the maternal parent, it is possible, in a reciprocal cross, to get two hybrids which are genetically identical but which have widely different seed sizes." Probably, in view of the context, the determination of seed size is not wholly by the maternal parent, as it would seem strange were the effect of hybrid vigor not exhibited in the embryo of squashes.

Murdoch (*9*), improving on Ashby's technique of dissection of the maize embryo, confirmed the results of Ashby as to weight of embryos but apparently overlooked Sprague's results, which first proved that

hybrids did not always have heavier embryos than their parents. He believed that one reason for the lack of accord between Ashby and his critics resulted from a failure to understand what is meant by the term "embryo." For his study, which was limited to embryo size, Murdoch removed the scutellum, the coleorhiza, and the endosperm.

It is true that the scutellum, coleorhiza, and endosperm do not become part of the mature corn plant, and for the purpose of calculating the rate of growth of the plant following germination these parts should not be considered as capital tissue. However, from the standpoint of the effect of hybrid vigor, each of these parts should respond alike to heterosis, as they are the products of fertilization. There is a cytological reason for discounting the response of the endosperm to cross-pollination, because the female parent plays a disproportionate part in the development of this tissue, furnishing two sets of genes to one from the male. In the case of the embryo tissue, comprising the scutellum, coleorhiza, and the embryo proper, both parents contribute equally, and indeed the scutellum and the coleorhiza are as much parts of the embryo as are the leaf and root primordia. They do not increase in cell number as the seedling expands and in a strict sense are not part of the capital. Their size, however, at the resting stage must be considered as much the result of heterosis as that of the primordia which are to become the next stages of the plant. It should be kept in mind that the cells of the mesocotyl (epicotyl), coleoptile, and indeed those of part of the leaves and roots, are formed before the resting period, and that the elongation of these organs, together with some part of their increase in dry matter in the early stages of growth, is in the nature of cell expansion. Roughly it might be estimated that half the increase in length of mesocotyls and coleoptiles results from the expansion of cells formed before the resting stage of the embryo.

Although the scutellum may not increase in dry weight with the growing plant, it is an indispensable part of the seedling, without which the embryo could not resume growth. This organ, therefore, should not be disregarded in considering the effect of hybrid vigor on embryo weight. Furthermore, it is quite likely that the weight of the scutellum increases with the resumption of growth after the resting period and that this weight is properly a part of the total dry weight of the seedling, even though it has not resulted from further cell division. The starch of the endosperm is not essential, as excised embryos will grow without it.

Ashby (*2, p. 1010*) gave data for the weights in milligrams of seeds, embryos, scutella, and total embryos for two parents and the F_1 and F_2 of maize, the seed of which was furnished by F. D. Richey. From the table, by subtraction, the endosperm weight can be derived. Taking the mean of the two parents as a base, it is found that the F_1 hybrid exceeds the parents in weight of grain by 14 percent, in weight of embryo by 58 percent, in weight of scutellum by 67 percent, and in weight of endosperm by 8 percent.

From the above data it is evident that the embryonic tissue represented by the scutellum increases in weight over the mean of the parents in the same order as does the embryo. The errors are such that the difference between these two parts of the embryo in percentage increase is not significant.

The relation of increase in weight of embryo to increase in weight of endosperm should throw some light on the nature of hybrid vigor. If dominant favorable growth factors are the sole explanation and dominance is complete, then the embryo and endosperm should show similar increases in weight, as the additional set of genes contributed by the female parent to the endosperm would have no more effect than the single set contributed to the embryo. On the other hand, if the favorable growth factors are not dominant, then the increase in weight of the embryo should be twice that found in the endosperm. This follows if it is assumed that each set of genes in both the endosperm and embryo produces an equal effect on weight, and further, that the genes derived from the two parents produce additive increases. Adding one set to the endosperm having unit effect would result in a 50-percent increase in weight, whereas a similar set added to the embryo would result in a 100-percent increase in weight.

Paddick and Sprague (*10*, *table 3*) give data that show an almost perfect agreement in the magnitude of percentage increase or decrease in weight of hybrid endosperms and germs. Only two real discrepancies were found, namely, hybrids A 12 × A 55 and A 25 × A 55. In the first of these hybrids the endosperm exceeded the female parent in weight by 34 percent, while the embryo showed only a 2.8 percent increase. In the second hybrid the endosperm exceeded the female parent in weight by 5 percent, while the embryo showed a 27.7 decrease. In their table 2, where 14 paired individual seed comparisons between hybrids and the female parent are shown, there are a number of wide differences between endosperm and embryo in their percentage increase in weight over the female parent. There is only one instance, however, where the hybrid germ shows a greater percentage increase in weight than the endosperm, and this is too small to be of significance.

Brink and Cooper (*6*) found in the case of alfalfa that the cells of the embryo increased in arithmetical progression for the first 6 days after fertilization. These authors also concluded that the rate of increase in number of cells in the embryo was no greater in crosses than in selfs. On the other hand, the nunber of nuclei in the endosperm during the same 6-day period increased exponentially, and the rate of increase in number was very much greater in crosses than in selfs. The authors point out that under these conditions hybrid embryos are accompanied by more advanced endosperm than inbred embryos. This circumstance might explain the greater size of hybrid embryos at the resting stage if it meant that greater endosperm activity resulted in better nourishment for the embryo. Seven plants were utilized in this study, and, although the crosses were between unrelated plants within the group, it is not clear whether the genetic backgrounds were such that heterosis was to be expected.

MATERIALS AND RESULTS

GERM WEIGHTS OF HYBRIDS AND PARENTS

Some of the seed stocks used by Ashby (*1*) in his original tests were furnished him by the writers and these have been used to check

his results. The cross was between an inbred line of Pipe, self-pollinated for 12 years, and an inbred line of Pawnee, self-pollinated for 20 years. Crossed and self- or sib-pollinated seeds were produced together on the ears of Pipe and separated by means of differences in aleurone color. Two crosses were tested, both having the same Pawnee parent but differing in that in one case the female Pipe parent was self-pollinated whereas in the other the Pipe parent was pollinated with a sib plant. No seed was obtained from the selfed Pawnee male parent. The results are shown in table 1.

TABLE 1.—*Mean weight of whole seeds and endosperm and embryo fractions for single plant pollinations*

Progeny	Air-dry weight of whole seed	Oven-dry weight of—		
		Whole seed	Endosperm	Embryo
SELFED OR SIBBED FEMALE AND COMPARABLE HYBRID SEED ON SAME EARS				
	Gram	*Gram*	*Gram*	*Gram*
Pipe 194×Pawnee	0. 2467±0. 0021	0. 2336±0. 0022	0. 2125±0. 0019	0. 0211±0. 0004
Pipe 194 selfed	. 2007± . 0016	. 1882± . 0014	. 1743± . 0015	. 0139± . 0002
Pipe 195×Pawnee	. 2112± . 0012	. 1986± . 0013	. 1812± . 0013	. 0174± . 0009
Pipe 195×Pipe 194	. 1709± . 0089	. 1598± . 0094	. 1481± . 0081	. 0117± . 0007
SELFED AND HYBRID SEED ON SEPARATE EARS				
Pawnee selfed	0. 2129±0. 0061	0. 1923±0. 0055	0. 1731±0. 0051	0. 0192±0. 0006
Pipe×Pawnee	. 2765± . 0028	. 2519± . 0026	. 2291± . 0031	. 0228± . 0006
Pipe×Pawnee	. 2902± . 0056	. 2635± . 0049	. 2387± . 0045	. 0248± . 0008
Pipe selfed	. 2955± . 0031	. 2662± . 0026	. 2459± . 0029	. 0203± . 0004

Where hybrid seeds are borne on the same ears with selfed seed the increase in weight of endosperm is 22 percent and in that of embryo 52 percent (table 1). These increases also are found where hybrid seed is borne on the same ear with sib-pollinated seed, the endosperm of the hybrid exceeding that of the inbred by 22 percent and the hybrid embryo exceeding the inbred by 49 percent.

Two other hybrids between these inbred lines were examined. In these two cases selfed seed was not borne on the ears with the hybrid seed. The comparison involves three plants of the inbred Pipe, two having been crossed with Pawnee and one self-pollinated. The results are shown in table 1.

Both hybrids had endosperms lighter in weight than that of their female parent, and the embryos were only 12 percent and 22 percent heavier, respectively, than that of the female parent. Grown to maturity, these Pipe × Pawnee hybrids far exceeded their inbred parents in yield.

A set of sister plants was used to test the increase in weight of plants at various periods after planting. These crosses, because of the small number of seeds, could not be used to test both germ weight and growth increase. Seed weights were recorded and are shown in table 2.

TABLE 2.—*Mean weight of whole seeds and estimated weight of embryo for single plant pollinations*

[Selfed female and hybrid seed on same lines]

Progeny	Air-dry weight of whole seed	Estimated dry weight of embryo	Progeny	Air-dry weight of whole seed	Estimated dry weight of embryo
	Gram	*Gram*		*Gram*	*Gram*
Pipe 181 selfed	0.199±0.003	0.0136	Pawnee 521 selfed	0.198±0.014	0.0178
Pipe 181 × Pawnee 524	.225± .006	.0187	Pipe 182 selfed	.306± .012	.0210
Pawnee 524 selfed	.205± .018	.0185	Pipe 182 × Pawnee 523	.313± .022	.0260
Pipe 192 selfed	.220± .008	.0151	Pawnee 523 selfed	.244± .013	.0220
Pipe 192 × Pawnee 521	.258± .011	.0214			

From the data presented in table 1, it is seen that the embryos of self- or sib-pollinated Pipe plants are 6.9, 6.8, and 6.9 percent of the air-dry weight of the seed; those of Pipe × Pawnee are 8.6, 8.2, 8.2, and 8.5 percent; and the weight of the embryo of the single Pawnee given is 9 percent of the air-dry seed weight. From other examples not given here of seed weights of sister ears of these lines for this year (1935) it can be safely assumed that these percentages hold reasonably well. They have been used, therefore, to estimate the approximate germ weights of the seeds grown to obtain information on the rate of increase in plant weight. The estimated weights, based on percentages of 6.85, 8.3, and 9, are given in table 2.

These hybrids are identical with those reported by Ashby, though made 4 years later. The parents, therefore, had four generations more inbreeding. The estimated increased weights of hybrid germs over those of the female parent of 37.5, 41.8, and 24.4 percent compare with sister ears actually weighed and presented in table 1 of 52, 49, 12, and 22 percent and with Ashby's (*1*) material of 18.5 percent.

The field experiment was planted May 20, 1935, in 11 Latin squares, 3×3, planted in three rows, with five seeds 2 inches apart in each hill. The choice of seed and blocks was random. Eliminating the variance due to rows, columns, and means of Latin squares did not improve the accuracy of the test, and since the differences between means of sorts are large, the use of a generalized error derived from the analysis of variance is of doubtful propriety.

The errors, therefore, are estimated from the deviations of the plants of each sort from the mean of that sort for a given sampling date.

The mean weights in grams of the entire plant for the several sampling periods are given in table 3.

TABLE 3.—*Mean oven-dry weight of plants of the inbreds Pipe and Pawnee and the F_1 hybrid between them for various periods*

[Planted May 20, 1935]

Date of sample	Oven-dry weight of entire plant		
	Pipe	Pawnee	F_1 hybrid
	Grams	*Grams*	*Grams*
June 5	0.145±0.009	0.177±0 10	0.274±0.015
June 12	.395± .029	.428± .042	1.213± .056
June 19	1.687± .188	2.126± .287	7.407± .480
June 26	4.638± .044	7.655±1.451	21.273±1.549

The rates of increase for the three periods of 7 days each are given in table 4, and also the significance of the differences in rate of increase between the hybrid and each parent.

TABLE 4.—*Mean rate of increase in dry matter for the inbreds Pipe and Pawnee and the F_1 hybrid between them and differences in rate of increase between hybrid and each parent*

RATE OF INCREASE [1]

Period	Pipe	Pawnee	Hybrid
June 5 to 12	2.73±0.26	2.42±0.27	4.42±0.32
June 12 to 19	4.28± .57	4.96± .83	6.11± .49
June 19 to 26	2.75± .31	3.60± .84	2.87± .28

DIFFERENCES IN RATE OF INCREASE

Period	Hybrid−Pipe	Diff./S.E.	Hybrid −Pawnee	Diff./S.E.
June 5 to 12	1.69±0.41	4.1	2.00±0.42	4.7
June 12 to 19	1.83± .75	2.4	1.15± .96	1.2
June 19 to 26	.12± .42	.3	−.73± .89	.8

[1] Ratio of later to earlier value, from data given in table 3.

It will be seen that for the first period (June 5–12) the rate of increase of the hybrid was significantly larger than that of either parent. In the second period, while the rates of increase were all larger than in the first period, perhaps due to better growing conditions, the superiority of the hybrid declined, becoming of somewhat doubtful significance. And in the third period all differences were without significance, the inbred Pawnee actually making a slightly greater increase than the hybrid. The results of this experiment support those of Ashby and Sprague, but it should be noted that the errors of rates are such that differences of less than approximately 30 percent cannot be established. A 30-percent difference in rate would be greater than necessary to produce most of the observed increases in size of hybrids over their parents, irrespective of any differences in initial weight of meristematic tissue.

The data suggest that whatever factors led to the production of heavier embryos before dormancy operated also in the early seedling period.

A second series of F_1 crosses between inbred lines was examined. These lines are wholly unrelated to the Pipe and Pawnee lines and were inbred for only seven generations before the crosses were made. All pollinations were controlled, but each crossed and selfed line involved many plants. The seed was bulked, and 10 samples of 10 seeds each were drawn from the thoroughly mixed stock. The mean weights of various parts of the seeds are shown in table 5.

TABLE 5.—*Mean weight of whole seed and endosperm and embryo fractions for multiple plant pollinations*

[Selfed and hybrid seed on separate ears]

Inbred lines and hybrids	Air-dry weight of entire seed	Oven-dry weight			Weight of embryo [1]
		Entire seed	Endosperm	Embryo	
	Gram	*Gram*	*Gram*	*Gram*	*Percent*
Line A	0.3092±0.0084	0.2772±0.0084	0.2597±0.0084	0.0175±0.0006	6.3
A×B	.3652± .0065	.3394± .0058	.3177± .0057	.0217± .0012	6.4
Line B	.1853± .0037	.1679± .0034	.1555± .0031	.0124± .0005	7.4
C×A	.1984± .0017	.1843± .0017	.1738± .0017	.0105± .0004	5.7
Line C	.2180± .0035	.1987± .0031	.1822± .0009	.0165± .0007	8.3
Line D	.2530± .0026	.2291± .0023	.2140± .0024	.0151± .0006	6.6
B×D	.1765± .0058	.1615± .0054	.1486± .0052	.0129± .0039	8.0
D×B	.2903± .0060	.2659± .0058	.2482± .0054	.0177± .0006	6.7

[1] Measured as percentage of dry weight of seed.

As the cross showing the greatest increase in germ weight (A×B) proved to have the highest field yield and that showing no heterosis effect (C×A) proved to have the poorest yield, there is some evidence in this experiment to support the hypothesis that heterosis as exhibited in germ weight will be reflected in field yield. However, it should be noted that all of these lines and crosses are much alike because they were derived from a single variety. This conclusion receives slight confirmation from the field yields of the reciprocal crosses (B×D and D×B), where the cross having the heavier germ had a slightly higher yield.

In the hybrids of Pipe and Pawnee, the greater increases in embryo weights relative to those of endosperm suggest that hybrid vigor is not solely the result of the interaction of complementary dominant factors favorable for growth. However, the data in table 5 support the contrary position. In these hybrids, where increases in weight of embryo were obtained, increases of comparable magnitude were found also in the endosperms. The percentage increase or decrease in weight of embryo and endosperm is shown in table 6.

TABLE 6.—*Percentage increase or decrease in weight of the endosperm and embryo of hybrid seed over that of the female parent and of the average of both parents*

Hybrid between inbred lines	Hybrid—female parent		Hybrid—average of both parents	
	Endosperm	Embryo	Endosperm	Embryo
	Percent	*Percent*	*Percent*	*Percent*
A×B	22.4	24.0	53.1	45.7
C×A	−4.6	−36.4	−21.3	−38.2
B×D	−4.4	4.0	−19.5	−5.8
D×B	16.0	16.6	34.4	28.5

The inbred lines are lettered, and it will be noted that hybrids B×D and D×B are reciprocals and that A×B and C×A have one parent in common. It should also be noted that the male parent of hybrid A×B is the female of B×D and the male of D×B.

Hybrids B×D and C×A had germs weighing less than the mean of their parents, and the germs of hybrid C×A actually weighed less than the germs of its female parent. Hybrid B×D does not differ significantly in germ weight from its female parent nor from the mean

of both parents. The differences between the other three hybrids and their parents in germ weights may be considered significant.

When tested with a common check, the crosses A×B and B×D both exceeded the check in pounds of grain per plant, the former by 0.14 pound and the latter by 0.13 pound. Both increases are significant, with probabilities of 0.002. The hybrid C×A, on the other hand, was below the check in yield of grain per plant by 0.09 pound. This decrease may also be considered significant, although the probability of its being a chance decrease is 0.03. The order of these three hybrids in their yields of grain follows that of their germ weights.

Hybrid D×B was not tested, but the following year a direct yield comparison was made between the reciprocals B×D and D×B. In this test D×B exceeded B×D in yield by 4 percent, but the yield test could not establish differences of less than 15 percent. The field yields, however, corresponded with the germ weights.

The results of the field tests of these four hybrids may be considered as at least not contradictory of Ashby's findings. In addition, the germ weights of these crosses confirm Sprague (*12*) and Paddick and Sprague (*10*), in that the weights of hybrid germs are not invariably heavier than those of their parents, although the resulting plants show hybrid vigor.

GERM WEIGHTS IN F_2

The early results on the relation of germ weight to subsequent yield stimulated investigation, and as a result of this enthusiasm, a study was made of the germ weights in the second generation of the hybrid A×B; the F_1 data were given in table 5.

In this study an effort was made to sort the seeds visually into germ sizes irrespective of seed weight. Five groups were made because of pronounced differences in seed shape. There were flat medium, flat large, round medium, round large, and small. The flat and round refer to seed shape; large, medium, and small, to germ size.

The ear proved to have the distribution shown in table 7.

TABLE 7.—*Distribution of the F_2 seeds of the hybrid A × B based on selection for germ size and seed shape*

Seed character	Seeds	Class	Seed character	Seeds	Class
	Number	*Percent*		*Number*	*Percent*
Flat medium	84	34.3	Small	33	13.5
Flat large	46	18.8			
Round medium	51	20.8	Total	245	100.0
Round large	31	12.6			

Twenty-five seeds from each of these groups were weighed individually for germ size, and the results are shown in table 8.

TABLE 8.—*Mean weight of whole seeds and endosperm and embryo fractions from the F_2 of hybrid A × B*

Seed group	Air-dry weight of entire seed	Oven-dry weight		
		Entire seed	Endosperm	Embryo
	Gram	*Gram*	*Gram*	*Gram*
Flat medium	0.1924±0.0032	0.1774±0.0029	0.1595±0.0027	0.01781±0.00048
Flat large	.2161± .0048	.2009± .0043	.1779± .0038	.02297± .00075
Round medium	.2083± .0082	.1934± .0076	.1737± .0074	.01974± .00064
Round large	.2255± .0071	.2086± .0066	.1866± .0061	.02197± .00100
Small	.1587± .0076	.1462± .0071	.1305± .0065	.01568± .00076

Unquestionably, the selection for germ size was effective, but it will be observed that it was simply an indirect selection for seed size. As a more direct measure of this relation, the correlation between germ weight and the dry weight of seed without the germ has been calculated separately for each of the five seed groups. These five coefficients are shown in the last column of table 9.

TABLE 9.—*Dry weight of germ and correlation with dry weight of seed within five classes in the F_2 of hybrid $A \times B$*

Seed class	Weight of germ [1]	Correlation (r) of germ weight with weight of seed without the germ	Seed class	Weight of germ [1]	Correlation (r) of germ weight with weight of seed without the germ
	Percent			*Percent*	
Flat medium	9.3	0.38	Round large	9.7	0.43
Flat large	10.6	.59	Small	9.9	.71
Round medium	9.5	.26			

[1] Measured as percentage of air-dry weight of seed.

The subgroup population of only 25 seeds is too small to attach significance to all of these correlations. Combining the five groups into a single population of 125 seeds on the assumption that the selection was not efficient enough to separate the groups, the correlation between seed weight (without the germ) and germ weight is found to be 0.614 with a regression of germ weight on seed weight of 0.083.

It is apparent from table 9 that the relative weight of the germ to the total dry weight (without the germ) does not differ appreciably between the seed groups.

The frequency distribution of germ weight is very irregular, with an ill-defined mode and a mean weight about 10 percent below that of the F_1. The weights ranged from 0.0087 to 0.0324 with a mean of 0.0194. A large part of this unsatisfactory distribution results from the variability of seed weight. When germ weight is corrected for the regression on seed weight, the distribution achieves greater regularity, as shown in figure 1. There is no evidence of bimodality.

In another study, not reported here, germ weights were recorded of Cuzco maize. The mean weight of the oven-dry seeds was 1.149 ± 0.021 gm., and the mean germ weight was 0.104 ± 0.003 gm. The germ weight, therefore, was 9.1 percent of the seed weight, which is about the proportion found for most mature seeds.[3]

The correlation of seed weight (without the germ) and germ weight in the Cuzco maize was $r=0.393$ with a regression of germ weight on seed weight of 0.06.

Ashby concluded there was no correlation between seed weight and germ weight because in his material the lines having the heaviest seeds were not the ones with the heaviest germs.

If endosperm weight and germ weight are controlled, at least in part, by the same genic interactions, as would appear to be the case

[3] In immature seeds the dry weight of germs comprises a smaller proportion of the total dry matter. The germs of seed 21 days after fertilization are approximately 6 percent of the total dry weight.

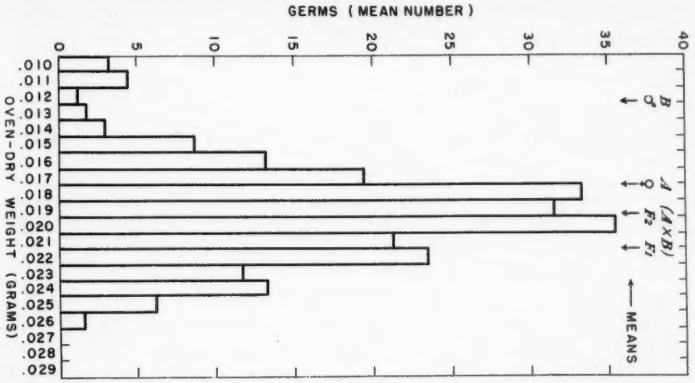

FIGURE 1.—Frequency distribution of germ weight for the seeds of the second generation of the hybrid A × B corrected for the regression of germ weight on seed weight.

from the data shown in table 6, then the weights of these two seed parts would not be independent. It would seem to be a safe inference that when hybrid seeds are heavier than those of their female parent the germs of such seeds will also reflect this greater weight. It is quite likely that interclass correlation would be low where diverse lines comprise the population, and where only a few lines are involved the correlation of seed weight and germ weight might even be negative. In the present examples it is evident that, given a population comprised of a mixture of Cuzco and any of the other sorts considered in this study, the correlation between seed weight and germ weight would be very close indeed.

GERM WEIGHTS IN EARLY STAGES OF GERMINATION

The weight of the germ in the resting stage must be determined by the rate of growth and the period elapsing between fertilization and dormancy. Given germs with equal rates of growth, their final weights must be equal unless their growing periods differ. If the resting stage could be eliminated, as it is in those mutations in maize designated as germinating seeds, or vivipary, the germ weight would not differ in character from seedling or plant weight. It follows that prolonging the growing period of the germ before the resting stage would result in adding to its weight, and since the resting stage can be eliminated it may be argued that when seeds are germinated in the dark, following their dormancy, the rate of increase in seedling weight with time corresponds to that which would have obtained had no resting period intervened. If this holds, then hybrids with small germs in the dormant stage should have slower growth rates in the early stages of germination than hybrids with heavy germs.

As a preliminary test of this point, three lots of seed from two of the F_1 hybrids shown in table 5, namely C×A and D×B, were germinated in the dark under identical conditions, and samples were withdrawn at the end of 24, 48, and 72 hours. From these samples the results given in table 10 were obtained.

TABLE 10.—*Mean oven-dry weight per seed of maize after various periods in a germinating chamber*

[F_1 hybrids (C × A) and (D × B) shown in table 7]

Period	Hybrid C × A			Hybrid D × B		
	Entire seed	Endosperm	Embryo	Entire seed	Endosperm	Embryo
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
Resting	0.1843±0.0017	0.1738±0.0017	0.01053±0.00038	0.2659±0.0058	0.2482±0.0054	0.01764±0.00057
24 hours	.1792± .0026	.1618± .0024	.01738± .00011	.2635± .0057	.2384± .0053	.02494± .00051
48 hours	.1798± .0024	.1618± .0022	.01810± .00028	.2762± .0061	.2500± .0056	.02619± .00086
72 hours	.1827± .0031	.1615± .0027	.02116± .00046	.2628± .0041	.2344± .0037	.02837± .00094

Considering the rate of increase in oven-dry weight of embryo, it is seen that C×A had a rate of 1.22 and D×B a rate of 1.17. The rates for the several periods are given in table 11.

TABLE 11.—*Mean rate of increase in dry matter of the germs of germinating seeds of the hybrids C×A and D×B for various periods*

Period	Hybrid C×A	Hybrid D×B
1st 24 hours	1.65±0.06	1.41±0.05
2d 24 hours	1.04± .02	1.05± .04
3d 24 hours	1.17± .03	1.08± .05
Entire period	1.22± .10	1.17± .07

It is seen that the hybrid C × A, which started with the less capital, had the more rapid rate of increase. The high rate in the first 24 hours accounts for most of this greater rate. If it is assumed that the germs of both hybrids were potentially of the same weight and that the observed difference in dormant weight resulted from an interrupted development prior to maturity in the case of C × A, then the rate of increase of the two hybrid germs might be compared for the periods when their initial weights were identical.

After 24 hours in the germinator, hybrid C × A had germs comparable in weight to those of hybrid D × B at the resting stage. The rate of increase for C × A for the period after the first 24 hours is 1.10.

None of these differences in rate, except that for the first 24-hour period, may be considered as significant.

RATE OF INCREASE IN WEIGHT OF GROWING GERMS

No data were collected showing the rates of growth of the embryos of these hybrids before dormancy. However, field studies have been made on other samples. The determination of rates of weight increase in germs on plants growing under field conditions offers many pitfalls because of the changing environment.

Two sorts of tests have been made with seeds maturing in the field. In one test, sections from ears pollinated at a known time were removed at various periods and rapidly dried. The remaining sections were removed at later periods, and the differences in germ weight between the periods were used to determine the rate of growth. In another set of tests, groups of whole ears pollinated on the same day were harvested at different dates, and the differences in germ size

between the early and later periods were used to determine the rate of growth. In both experiments the ears were of the same stock and the pollen used was composited from a number of sister plants.

The first method introduces a possible error if the seeds at the top half of an ear have smaller germs than those at the bottom half. The error from this source may be reduced by using for germ weights the seeds nearest the cut half in each case. However, for reasonable populations of germs, several rings of seeds must be used, so that even under ideal conditions many of the seeds on the separate ear sections are widely separated. Further, it often happens that on the section of the ear left to mature on the plant the seeds near the cut end either fail to attain a dormant stage or else this is of short duration, as many of them germinate before harvest.

The second method, as well as the first, introduces the unknown element of environment, as conditions for germ growth may change so that the observed differences may result, at least partly, from this cause. In the second method also there is introduced the element of genetic variation between plants as well as any differences of environment resulting from the fact that the plants occupy different locations.

The results obtained by these two methods are shown in table 12.

TABLE 12.—*Mean daily rates of increase in germ weights of seeds developing on field-grown plants*

Part of ear and period on which rates were based	Daily rates of increase in germ weights of seeds on—		Part of ear and period on which rates were based	Daily rates of increase in germ weights of seeds on—	
	Ears pollinated July 27	Ears pollinated August 1		Ears pollinated July 27	Ears pollinated August 1
Ear sections:			Whole ears:		
Aug. 21 to 23	1.135±0.039	1.327±0.090	Aug. 23 to 26	1.004±0.029	1.084±0.047
Aug. 23 to 26	1.072± .039	1.064± .021	Aug. 26 to 28	1.162± .025	1.192± .059
Aug. 26 to 28	1.082± .081	1.163± .046			
Aug. 28 to 30	1.056± .011	1.059± .041			

The errors show how unsatisfactory these methods are. There is little agreement between the results obtained on whole ears and on sections where the pollinations were made on July 27, whereas the agreement between these two methods is very good for the pollinations made on August 1. The study would have to be greatly extended before it could be determined whether or not the rate of increase in weight at these early stages was constant.

In conclusion, it may be pointed out that these early rates are of the same order of magnitude as those found for the first 3 days of germination.

GERM WEIGHTS OF VARIOUS SEED TYPES

On the theory that onset of dormancy interrupts the growth of the embryo it was thought possible that where sweet and starchy seeds developed upon the same ears the sweet seeds would have heavier embryos than starchy seeds, because sweet kernels retain a high moisture percentage longer than starchy seeds.

A self-pollinated ear segregating for sweet from a progeny repeatedly backcrossed to starchy until 93.75 percent of the genes were

common to both seed types was examined. This ear gave the weights shown in table 13, ear 1.

TABLE 13.—*Mean weight of whole seeds of various types, and endosperm and embryo fractions*

[Seeds of contrasting types borne on same ear]

Ear No. and seed type	Air-dry weight of whole seed	Oven-dry weight of whole seed	Oven-dry weight of endosperm	Oven-dry weight of embryo
Ear 1:	*Gram*	*Gram*	*Gram*	*Gram*
Starchy	0.3153±0.0052	0.2929±0.0049	0.2788±0.0051	0.01409±0.00051
Sweet	.2569± .0057	.2389± .0063	.2065± .0068	.03240± .00195
Ear 2:				
Starchy	.4691± .0085	.4333± .0080	.3888± .0072	.04449± .00128
Sweet	.3368± .0099	.3084± .0089	.2728± .0087	.03558± .00120
Ear 3:				
Horny	.3257± .0039	.3045± .0038	.2799± .0036	.02456± .00064
Waxy	.3264± .0045	.3040± .0042	.2807± .0043	.02333± .00081
Ear 4:				
Horny	.3533± .0030	.3308± .0030	.3057± .0032	.02505± .00079
Waxy	.3377± .0037	.3147± .0034	.2929± .0035	.02181± .00067
Ear 5:				
Flinty	.2508± .0021	.2374± .0022	.2177± .0022	.01964± .00064
Floury	.2479± .0023	.2315± .0024	.2094± .0196	.02221± .00066
Ear 6:				
Blue	.2631± .0021	.2409± .0016	.2197± .0021	.02118± .00098
Red	.2678± .0042	.2452± .0038	.2178± .0042	.02740± .00076
Ear 7:				
Yellow	.1849± .0013	.1726± .0011	.1534± .0011	.01927± .00025
White	.1812± .0018	.1684± .0017	.1506± .0018	.01777± .00033

The difference in weight of embryo is clearly significant and suggests that the slower maturation of sweet kernels, as indicated by their higher moisture content, may afford the germ a longer growing period before dormancy.

A second ear was examined from a similar but unrelated backcrossing experiment where 87.5 percent of the genes were common to both seed types. In this instance the seeds did not result from self-fertilization but were from crosses between sibs. The weights are given in table 13, ear 2.

In this instance the embryos of sweet seeds are most certainly lighter in weight than those of starchy seeds on the same ear, a result which contradicts the hypothesis of a longer growing period for the embryos of sweet seeds.

A similar study was made with respect to germ weights in horny and waxy seeds on the same ears where because of repeated backcrossing most of the genes were common to both seed types. The weights are given in table 13, ears 3 and 4.

Insofar as these two ears are useful for generalization, it appears that when the female gametes are all waxy and the male is heterozygous (ear 4) the germs of nonwaxy seeds are significantly heavier than those of their waxy sibs. The difference in weight of germ is greater than the corresponding difference in weight of endosperm. When the female is heterozygous (ear 3), the endosperm of the waxy seeds is actually, though not significantly, heavier than that of the horny seeds on the same ear. The germs of the waxy seeds, however, are lighter in weight, though the difference of 5 percent is not significant.

There is no evidence from either of these ears that the waxy type of endosperm provides a longer growing period for the germ before the onset of the resting stage.

A fifth ear was examined, where the contrasting seed characters were flinty-floury. This ear was from an Indian variety inbred for only two generations. The observed weights are given in table 13, ear 5.

This result might be taken as an indication that the physical character of the endosperm affected germ growth. However, two other ears were tested where the differences in seed type were colors and the endosperms were alike in texture.

The first of these is an Indian corn inbred for several generations but segregating for blue and red aleurone (*Pr, pr*). The observed weights are given in table 13, ear 6. In this case the embryo weight of the seeds having the recessive color class exceeds that of the dominant seed group by almost 30 percent.

The second of these color groups involved yellow and white endosperms. The observed weights are given in table 13, ear 7. In this instance the difference in weight of embryo between the two seed classes is also significant, but the recessive seed class has the lightest embryos.

Although there is little likelihood that heterosis is involved, the differences in germ weight between seed classes of the same ear are of the same order as those found between hybrids and their inbred parents and in some instances are of such a magnitude that they should result in wide differences in mature plant size, unless there is a compensating difference in growth rate.

From these data, accumulated for the several seed classes paired on the same ears, it is not possible to formulate conclusions other than that significant differences in germ weight are to be found in various seed groupings, even where most of the gene background is uniform for the classes.

SUMMARY AND CONCLUSIONS

The results reported by Sprague (*12*) are confirmed. Hybrid vigor is often, though not invariably, reflected in the dry weights of the resting embryos of maize. However, in those cases where the hybrid embryos are lighter in weight than those of their parents the plants grown from them exceed the parents in size.

The rate of increase in dry matter following dormancy is greater in hybrids than in their parents in the early stages but the greater rate is not maintained to maturity.

Seed groupings based on endosperm texture and color but with uniform genic backgrounds disclose differences in germ weights.

The increase in weight of embryo relative to the increase in weight of endosperm offers equivocal evidence on the hypothesis that hybrid vigor results from the interaction of complementary dominant factors favorable for growth.

The possibility remains that the measure of heterosis afforded by the germ weight of hybrids, in comparison with that of their parents, can be used as a preliminary evaluation of a series of crosses. The data available at present are inadequate to determine just how useful this criterion may be and, of course, the dry weight of the ultimate plant is only one element governing the choice of hybrids. If germ weights prove of value, the tedious operation of removing dormant germs apparently can be obviated by utilizing the early stages of germination.

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RELATION OF NAVAL STORES YIELDS TO FREQUENCY OF CHIPPING[1]

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INTRODUCTION

The common commercial practice in wounding, or chipping, longleaf pine (*Pinus palustris*) and slash pine (*P. caribaea*) to obtain gum from which naval stores are distilled, is to make the wounds, or streaks, at weekly intervals from March to November, continuing from season to season until the series of streaks, or face, is run up to a practical working limit of about 9 feet.

Early in the history of the industry, operators learned that the yield of gum so obtained is in some way related to the weather, particularly to the temperature, and that the yield is much less in the cooler winter months than in the warmer summer ones. Some of the more progressive producers observed that fair gum yields could be obtained by chipping during periods of warm weather in the winter. A few operators, in more recent years, have made a practice of double chipping, that is, chipping twice a week during the summer. These variations from customary weekly and seasonal chipping practice are used mainly to increase the total production per season or year. Some of the winter chipping, however, is done to prevent the formation of unproductive pitch-soaked wood, which forms when a turpentined tree remains unchipped for several months.

There are other reasons for departing from customary chipping schedules. If an operator finds it desirable for various reasons to lengthen or shorten the utilization period, he chips less or more than the customary number of streaks per season, thereby reducing production per season and distributing it over more seasons or increasing production over fewer seasons. This calls for a somewhat flexible labor supply as well as availability of woods work other than turpentining. Labor conditions, in fact, are often responsible for changes in chipping schedules. In times of labor scarcity, streaks will be missed during the regular season, whereas a plentiful supply of labor often results in more frequent chipping than usual, especially during the hottest months. Chipping frequency thus obviously depends, in part at least, upon economic factors. Again, the importance of considering silvicultural and forest-management objectives is becoming apparent, and these factors are already beginning to overshadow the economic factors. In thick stands of timber, for example, the trees least desirable for the final crop can be chipped frequently for quick naval stores exploitation prior to thinning. Likewise, it is more profitable to exploit saw timber quickly for gum prior to logging than to prolong the turpentining and expose such timber for several more years to the risks of fire, wind, and insects.

[1] Received for publication July 9, 1941.

Because the reasons for employing different chipping-frequency schedules are so numerous and varied, there has been in recent years considerable investigation of the effect of frequency of chipping on gum yield. In Germany, such names as Münch, Schierlinger, and Cieslar are primarily associated with these investigations; in France, Oudin; in Sumatra, Buys; in Russia, Ivanov; in Spain, Iturralde; and in the United States, Shorger, Herty, and Wyman. Detailed information, however, on the relations between weather and gum flow is for the most part lacking. The comprehensive basic study of Harper and Wyman [2] on this relationship formed the first thorough basis for evaluating the effect of chipping frequency on yield. They state:

> The study has afforded a basis for estimating, for any given average air temperature, the gum yield to be expected from a streak and the optimum interval between chippings. In general, the temperatures of the months of November to March, inclusive, are found suitable for semimonthly chipping, those of July and August for semiweekly chipping, and those of the remaining months for weekly chipping, for longleaf pine. A similar schedule is applicable to slash pine, but for this species the optimum chipping intervals are in general longer than for longleaf pine.

Because they were working almost entirely with weekly chipping, howeve , they were not able to determine how long or how much gum a streak will produce if allowed to run until it ceases entirely. Furthermore, although they demonstrated conclusively that season or temperature must be the deciding factor in varying the interval between chippings, they did not determine actual yields or the effect of frequent and continuous chipping on the vitality of the timber.

Recognizing the importance of chipping frequency and the determination of optimum intervals between chippings, the Southern Forest Experiment Station started in the spring of 1934 on the Olustee Experimental Forest in northeast Florida an experiment to determine for longleaf pine (1) the effect of different frequencies of chipping on the yield capacity of a tree, and (2) the comparative total yields of gum from varying frequencies of chipping. At the same time another study was begun to determine for both longleaf and slash pine (1) the total yield of gum per streak for different months of the year when the streak is allowed sufficient time to run dry, that is, to cease yielding gum, and (2) the time required for a streak to cease yielding gum in different months.

Details of the second study have been reported briefly elsewhere.[3] They will not be recounted here, but the summarized results will be combined herein with the results of the first study in discussing optimum intervals between chippings.

FIELD WORK

In establishing the frequency-of-chipping study, 66 trees were selected, on the basis of diameter at breast height [4] and growth rate (number of rings in last radial inch), from a rather uniform, well-stocked pure stand of round second-growth longleaf pine approximately 35 years old. The trees then received the first streak about 7 inches from the ground. Galvanized tins and flat-bottom cups

[2] HARPER, V. L., and WYMAN, LENTHALL. VARIATIONS IN NAVAL-STORES YIELDS ASSOCIATED WITH WEATHER AND SPECIFIC DAYS BETWEEN CHIPPINGS. U. S. Dept. Agr. Tech. Bul. 510, 35 pp., illus. 1936.

[3] LIEFELD, T. A. HOW LONG WILL A STREAK YIELD GUM? Naval Stores Rev. 48 (50): 10, 14, illus. 1939.
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[4] Measured 4½ feet above ground.

were installed in shallow broadax incisions. Streaks ½ inch high and ½ inch deep were chipped on schedule throughout the duration of the experiment by the regular chipper assigned to the experimental forest.

During a preliminary period, April 9 to May 29, 1934, 8 streaks were chipped on all the trees at weekly intervals. Total yield per streak was recorded for each tree to obtain an indication of individual yield capacities. The trees were then assigned permanent numbers and grouped into 6 treatment groups of approximately equal potential yield. Each group consisted of 10 trees and 1 extra to replace any which might be killed or weakened by agencies other than those studied.

The three chipping schedules shown in table 1 constituted the experimental treatments, which were begun June 4, 1934. Treatment 1 represented the customary seasonal chipping practice. Treatment 2 was slightly heavier; the intervals between chippings were varied according to seasons, and winter chipping was included. Treatment 3 was considerably heavier than 2 during the summer period. When the treatments were selected, it was anticipated that treatment 1 would be the check by which the others would be compared; that treatment 2 would represent the optimum in the way of a varying frequency schedule; and that treatment 3 would be too frequent for sustained yield.

The 66 trees selected permitted 2 replications of each treatment of 10 trees. One group within each treatment was treated on a certain day and the other group on the following day, in order to obtain more observations of air temperature. Chipping and gum-weighing schedules were constantly posted so that the plans of the study could be adhered to rigidly. Field inspections were made every week and notes taken that might be of value in subsequent office analyses.

TABLE 1.—*Number of streaks chipped monthly at regular intervals, in three experimental treatments* [1]

Month	Treatment 1	Treatment 2	Treatment 3
January	0	2	2
February	0	2	2
March	2	2	4
April	4	4	4
May	4	4	8
June	4	4	8
July	4	8	12
August	4	8	12
September	4	4	8
October	4	4	4
November	2	2	2
December	0	2	2
Total number of streaks	32	46	68

[1] Treatments 1, 2, and 3 represent respectively common practice, medium work, and heavy chipping. Figures given are for 4-week months and hence only approximate actual chipping, which is by calendar weeks.

The 24-hour yield collected in the cups and the total yield for each streak were weighed, whenever the schedule called for a weighing, between 7:30 and 9:30 a. m., on a Harvard balance scale carried from tree to tree. A transparent hood effectively shielded the scales from excessive air movements. Before the cups were weighed, however, the scrape, or hardened gum adhering to the face, was added to the cups. At the same time, as much as possible of the trash

and rain water present was carefully removed or poured off. This field procedure of chipping and weighing according to schedule continued for the duration of the study.

At the end of December 1936, a total of 206 streaks had been applied in treatment 3 and a height of approximately 9 feet had been reached. These faces were considered worked out and were discontinued. Those in the other treatments, however, were still operable; only 92 streaks had been chipped in treatment 1, and 143 in treatment 2. It was decided to work out the first or front faces of treatment 2 with a total of 206 streaks, at which time it would be abandoned entirely. This happened early in May 1938. Chipping was discontinued on the front faces of treatment 1 at the same time as in treatment 3, even though 114 additional streaks, or nearly 4 more years of treatment, could have been applied. It was deemed more desirable to begin simultaneously a second or back face in both treatments 1 and 3 early in 1937, in order to provide data for a comparison of back-face yields in treatments 1 and 3. Front-face yields from treatment 1 were extrapolated from the time the faces were discontinued until they would have been worked out. Cups were installed for back faces in treatments 1 and 3 on March 29 and April 5, 1937, respectively. These dates also marked the beginning of the treatments. On October 24, 1938, the sixty-fourth streak, or the last of two full seasons, was chipped in treatment 1. Chipping continued in treatment 3 for a total of 137 streaks until the end of the year, when all field work was discontinued.

ANALYSES OF DATA

Two separate analyses of the data were made, one to determine the effect of the three treatments on the yield trends of the groups, and the other to compare the actual yields from the treatments. The variables, selected as being primarily important in such a study as this, were the same in both analyses but expressed in different terms. Gum yield of a 10-tree group, in whole grams, was the dependent variable (X_1). In the first analysis it was the yield recorded for the first 24 hours after chipping; in the second analysis it was the total yield per streak. The independent variables were temperature (X_2) and time (X_3). The temperature, which in both analyses was expressed as maximum air temperature (in degrees Fahrenheit) that occurred on each day of chipping, was obtained from the daily thermometer and thermograph readings at a standard Weather Bureau shelter on the experimental forest, about 1,000 feet from the scene of the study. Time in the first analysis was expressed as the number of days or weeks elapsed since treatments began, and in the second analysis as the number of the streak.

Gum yields in the first 24 hours were found to be no more effective than total yield per streak in expressing the effect of treatment on the vitality or yield capacity of a 10-tree group. Only the final results, therefore, of the first analysis are presented in figure 1, showing for the different treatments the net trend of yield (effect of temperature largely removed) since treatment began, or the effect of treatment on yield capacity. The solid, dashed, and dot-dashed portions of the curves are actual trends computed from the data, whereas the dotted portions are extrapolations up to the time the faces would have been

worked out if an equal number of streaks had been applied in all treatments. Approximately half the front-face trend of treatment 1 has been extrapolated.

In the second analysis—comparison of the yields from the different treatments—interest centers around the partial regressions of yield on

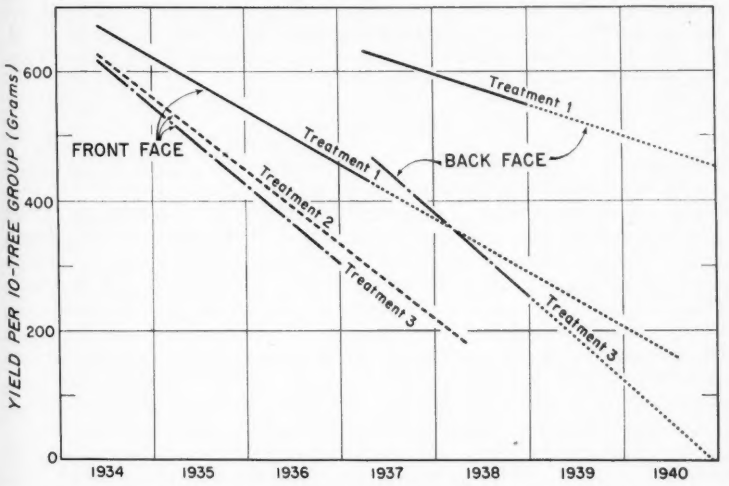

FIGURE 1.—Results of the 24-hour gum-yield trends of treatments 1, 2, and 3. The dotted portions of the curves are extrapolations as explained in the text.

time, expressed as number of streak, with the effect of temperature again largely removed. Multiple linear regressions of yield on temperature and number of streak in the form,

$$X_1 = a_{1.23} + b_{12.3}X_2 + b_{13.2}X_3,$$

were computed separately by the least-squares method [5] for front and back faces and also for each treatment. First approximations are shown in table 2. Curving these values by successive approximations

TABLE 2.—*Multiple linear regressions and other pertinent data for curves showing trend of total yield per streak*

Face and treatment No.	Regression	Means [1]			Number of observations	Multiple correlation coefficient
		X_1	X_2	X_3		
Front face:						
1	$X_1 = 13.226X_2 - 1.439X_3 - 205.277$	882.330	87.415	47.602	176	0.489
2	$X_1 = 2.704X_2 - 2.603X_3 + 779.456$	734.274	84.240	104.859	405	.670
3	$X_1 = 3.701X_2 - 1.657_3 + 555.593$	700.121	86.616	106.259	398	.564
Back face:						
1	$X_1 = 11.630X_2 + 3.184X_3 - 158.324$	948.359	86.258	32.500	128	.710
3	$X_1 = .605X_2 - .901X_3 + 634.229$	624.179	86.179	69.048	273	.256

[1] X_1=Total gum yield per streak per 10-tree group, in grams; X_2=maximum air temperature, on day of chipping, in degrees Fahrenheit; X_3=streak number.

[5] EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London 1930.

allowed adequately for temperature and at the same time evolved curvilinear partial regressions of yield on streak number which would follow the actual data more closely than the linear (fig. 2).

In figure 3 the partial regressions of yield on temperature are shown as corrections to the regressions of figure 2. Values were then read from the partial regressions of yield on streak number to form curves of cumulative yield over streak number. These cumulative-yield curves were superimposed for easier comparison, as shown in figure 4, *A*. The same data presented on the basis of calendar time rather than streak number are given in figure 4, *B*.

DISCUSSION

Whenever gum yield from such chipping specifications as were used in this experiment is plotted over a time interval of 3 or more years, a definite downward trend will always be observed. To a small extent this is due to the gradual decrease in face width as chipping progresses up the tree to conform with the natural taper of the stem, but primarily to a gradual decrease in vigor or yield capacity of the tree. This decrease varies, of course, with the intensity of the turpentining, a phenomenon which has been observed and measured repeatedly in numerous naval stores studies, but both face taper and decline in vigor affect trends more markedly after the third year. It is to be expected, as verified by the slopes of the curves in figure 1, that the rate of decline would be greatest for treatment 3 (both the front- and back-face work) and least for treatment 1. Had treatment 1 continued until the faces were worked out, its downward trend undoubtedly would have been greater.

The back-face trends of treatments 1 and 3 reflect somewhat the same situation. Had the front faces of treatment 1 been worked out by continuing the treatment through 1940, before installing back faces, the rate of decline of back-face trends probably would have been greater and the general level somewhat lower. It should be pointed out that the general levels of the front-face trends are relatively unimportant. That they are not the same merely indicates that the preliminary 8-week chipping period was not sufficiently long to establish reliable yield capacities for the individual trees in the study, and that the assignment of trees into groups of equal yield capacities was not entirely successful. The slopes of these trend curves, however, are of major importance in that they portray the drain in vigor resulting from the different treatments. In the back-face curves, both the levels and the slopes are important, and would have been even more so if the front faces of treatment 1 had been worked out prior to back-facing. It is apparent, nevertheless, from the rapid rate of decline of treatment 3 during the period of back-facing, that timber which is to be chipped with the frequency of treatment 3, and probably even that of treatment 2, should be allowed to rest for at least 1 year before back-facing is begun. There does not appear to be sufficient difference in the effects of treatments 2 and 3 on the yield trends to prohibit, on the basis of decrease in vigor alone, such frequent chipping as in treatment 3.

Figure 4, *A*, in which readings from figure 2 have been converted into cumulative yields and plotted over streak number, shows that when comparisons of total production from front faces are made, and when the same total number of streaks has been chipped for each

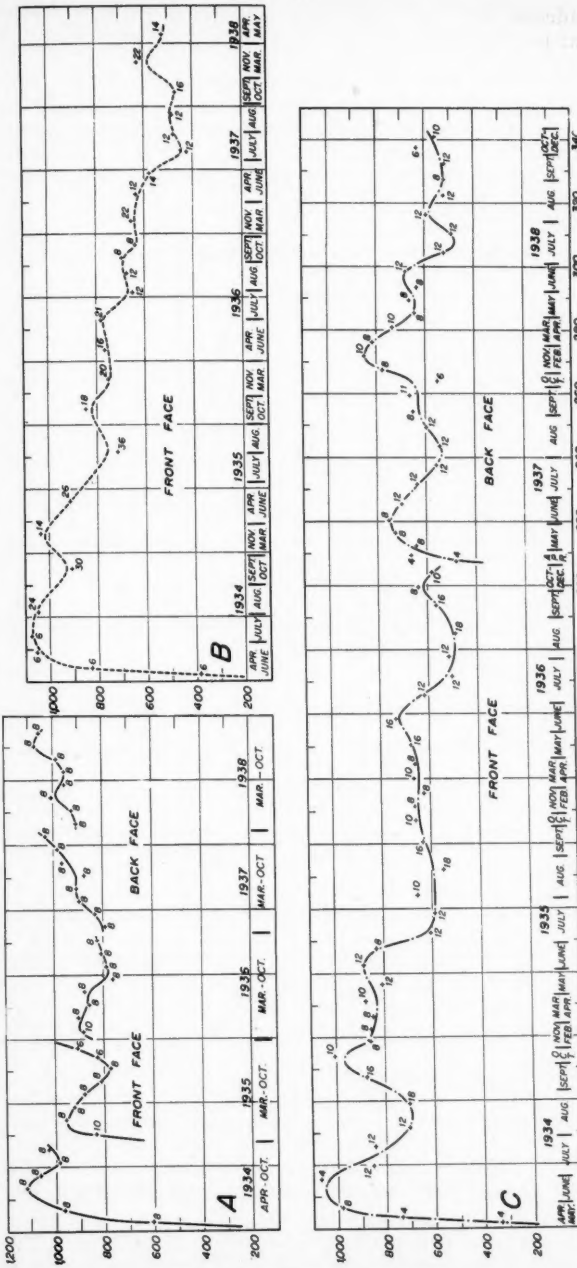

FIGURE 2.—Partial curvilinear regressions of total gum yield per streak on streak number: *A*, Treatment 1; *B*, treatment 2; *C*, treatment 3.

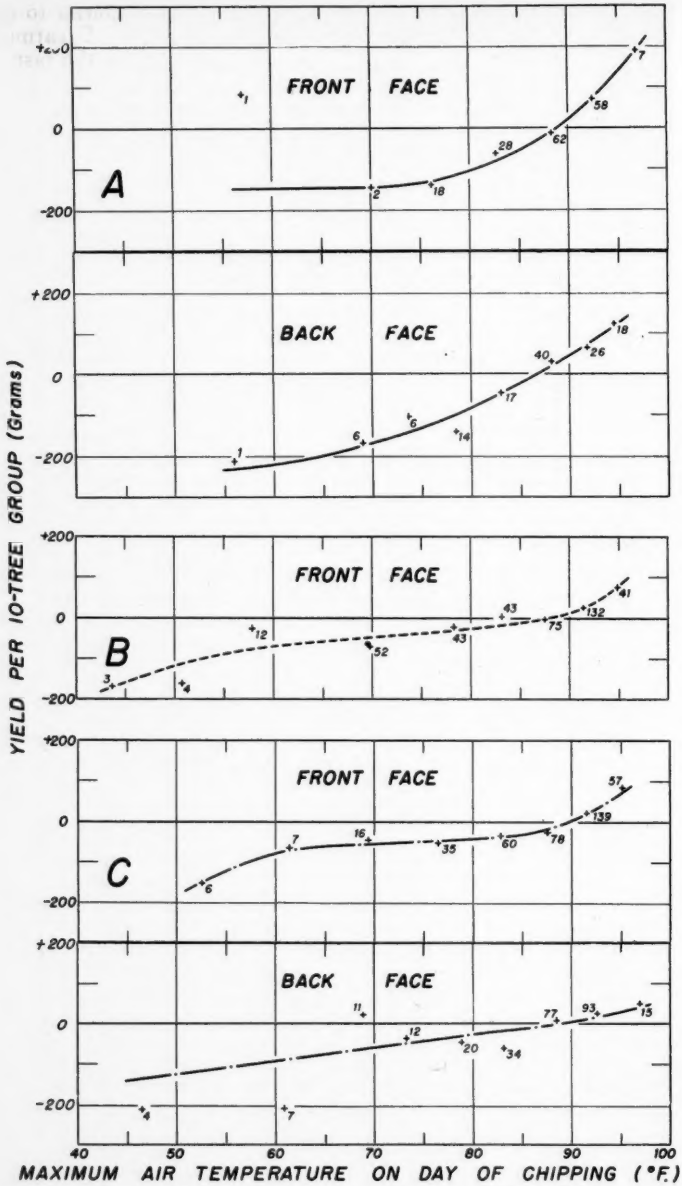

FIGURE 3.—Partial curvilinear regressions of total gum yield per streak on temperature, as corrections to figure 2: *A*, Treatment 1; *B*, treatment 2; *C*, treatment 3.

treatment, treatment 3 is approximately 10 percent inferior to treatment 1 and about 5 percent inferior to treatment 2. Treatment 2 is slightly superior to treatment 1, except possibly for the last year of work, i.e., after about streak No. 160. This superiority of treatment 2 is problematical, however, since the cumulative-yield curve of treatment 1 had to be extrapolated after streak No. 92. Obviously such frequent chipping as in treatment 3 hardly would be expected to surpass less frequent work, when comparisons are made on the

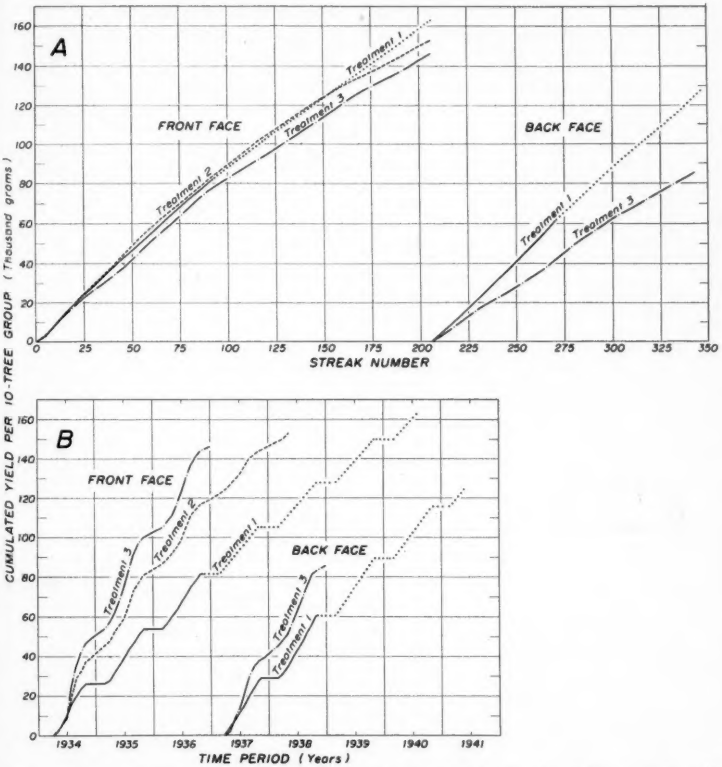

FIGURE 4.—Comparison of total gum yield per streak trends of treatments 1, 2, and 3: *A*, On the basis of streak number; *B*, on the basis of year of work.

basis of total yield per streak or cumulative yield, because the time during which most of the streaks are allowed to flow is diminished. The advantage of frequent chipping, for certain objectives at least, lies in greater production during a given length of time or, conversely, a shorter period of time to produce a given amount of gum. This advantage in time is clearly demonstrated in figure 4, *B*, although it is evident that total production from a face is diminished somewhat by heavier work.

In determining the effectiveness of any chipping-frequency schedule,

information is needed as to the rate and length of time a streak chipped at different seasons of the year will yield gum when allowed sufficient

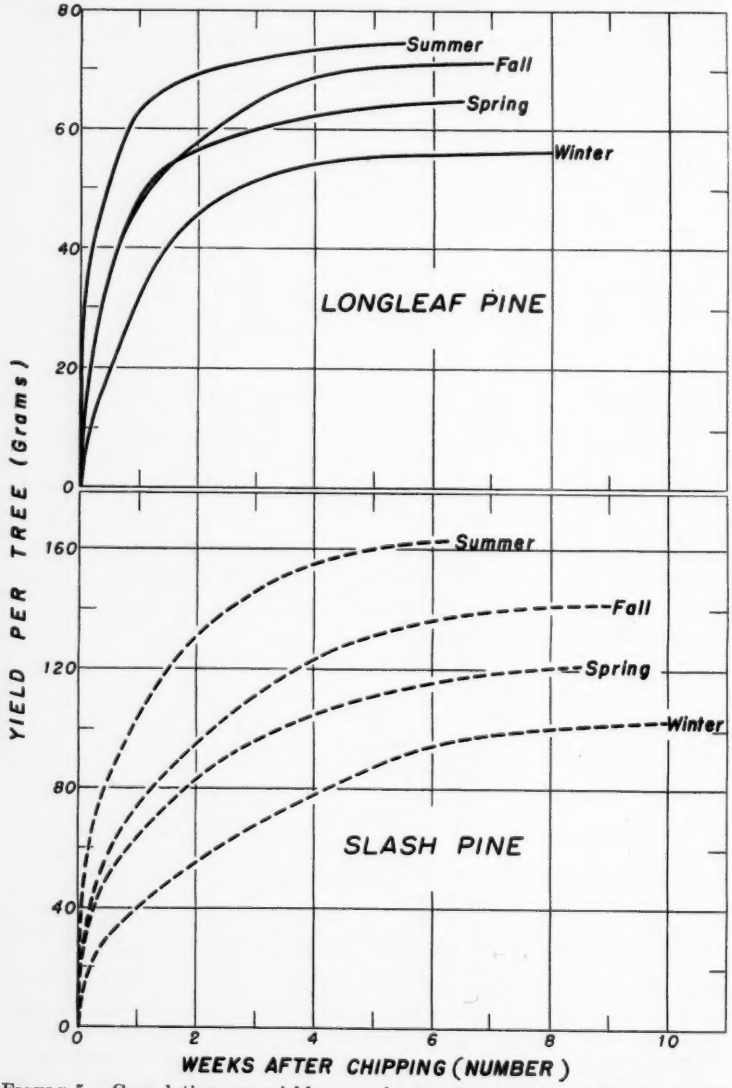

FIGURE 5.—Cumulative gum-yield curves for streaks chipped at different seasons of the year and allowed to run dry.

time to cease yielding entirely. Data on total yield per streak, which although not representative are at least indicative, are presented in figure 5 and table 3 for both longleaf and slash pine, as supplied by

the second study already mentioned. Values read from figure 5 at abscissa-axis values 1, 2, 3, and 4 are the basis for table 3.

TABLE 3.—*Relative gum yields from streaks chipped at different seasons of the year and allowed to flow for 4 weeks*

Species and season of streaking	First week —ratio to first week of summer	Ratio of other weeks to first week		
		Second week	Third week	Fourth week
Longleaf pine:	*Percent*	*Percent*	*Percent*	*Percent*
Summer	100	9	4	3
Spring	77	16	7	6
Fall	73	22	15	9
Winter	50	44	17	8
Slash pine:				
Summer	100	26	15	9
Spring	60	36	21	15
Fall	69	33	19	18
Winter	37	40	30	27

When the study was started it was anticipated that the rate of gum yield would be practically the same from frequent as from infrequent chipping, thus yielding appropriate data for detailed comparisons of different frequency schedules. Data from the study showed, however, that gum flow is more sluggish when the streaks are chipped at long intervals than when chipped weekly or more frequently. The curves of figure 5, based on a chipping interval of 6 to 10 weeks, do not portray the more rapid rise of curves based on weekly chipping. Nevertheless, a few general conclusions can be drawn from the figure. It will be noted from the termini of the individual curves that slash pine consistently flows somewhat longer than longleaf pine and that for both species the time required for a streak to cease yielding gum is greatest in winter (December 22 to March 21) and progressively less in fall (September 22 to December 21), spring (March 22 to June 21), and summer (June 22 to September 21).

The shapes of the curves show that, after the second or third week, yields drop off more rapidly in hot than in cold weather. Evidently weekly chipping during the winter would be poor practice because too much potential yield would be lost from each streak. On the other hand, it would be equally poor practice to permit a streak to run for 6 or 8 weeks in the summer because too small a production to be profitable would result.

SUMMARY AND CONCLUSIONS

In this study of the effects of different frequencies of chipping on the yield trends and total yields of longleaf pine turpentine timber, three treatments were applied, approximating customary seasonal chipping practice, moderate year-round practice, and heavy year-round practice. The gum yield during 24 hours and the total yield per streak were recorded from two 10-tree groups in each treatment, for approximately 4 years, together with the maximum air temperature on the days of chipping.

Comparisons of the partial linear regressions of yield on time, after allowing for a curvilinear effect of temperature, brought out the fact

that neither moderate nor heavy year-round chipping causes excessive drain on the vitality of the turpentined timber.

Comparisons of cumulative-yield trends compiled from curvilinear partial regressions of yield on time, after allowing for a curvilinear effect of temperature, made clear the advantages of frequent chipping when rapid exploitation and high annual or seasonal production are desired. It was evident, however, that chipping frequencies should not exceed three times a week during the summer nor every other week during the winter.

Where long working life of the same faces or trees is considered more important than high production per year, the study points to the schedule of semiweekly chipping in the summer, weekly in the spring and fall, and semimonthly in the winter as recommended in 1936 by Harper and Wyman. Even in the most conservative work, however, loss of gum yield would result if streaks were chipped less frequently than every 2 weeks in the summer and every 6 to 8 weeks in the winter.

If for any reason varying intervals between chippings are out of the question, because of conditions existing in an operation, the results obtained would indicate the desirability of weekly chipping from March to November and chipping every other week during the winter, or, for longer working life, a rigid schedule of chipping every other week throughout the year.

VIABILITY OF EGGS OF THE SWINE THORN-HEADED WORM (MACRACANTHORHYNCHUS HIRUDINACEUS) [1]

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INTRODUCTION

Although the thorn-headed worm (*Macracanthorhynchus hirudinaceus*), which parasitizes swine, is of considerable economic importance according to Schwartz,[2] there is little available information concerning the effect of environmental factors on the eggs. Therefore, an investigation was undertaken to ascertain the effects of (1) high temperatures both instantaneously applied and sustained for 10 minutes, (2) freezing in water and in a dried condition, (3) continuous drying, (4) alternate wetting and drying, and (5) ultraviolet irradiation. The investigation was carried out from July 1938 to November 1939 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

MATERIAL AND METHODS

Eggs [3] used in the tests herein described were obtained from gravid female worms collected post mortem from infected swine and dissected within 24 hours after their removal from the host animal. The eggs were first concentrated in cone-shaped sedimentation glasses and then washed at least 10 times in succession by filling the cones with tap water, allowing the eggs to sediment for 20 to 30 minutes, and then discarding the supernatant fluid, which contained some incompletely developed eggs and pieces of worm tissue. After the washing process was completed, the eggs were stored in tap water at temperatures of 2° to 5° C. until used.

The criterion for ascertaining the viability of the eggs was the capacity of the contained larvae to infect final instar grubs of the green June beetle (*Cotinis nitida* (L.)). Under experimental conditions the larval stages of this beetle are capable of serving as intermediate hosts of thorn-headed worms. The grubs were collected from soil to which hogs had not had access for 8 to 10 years, a procedure which reduced to a minimum the chances of extraneous infection of the grubs with this parasite. No thorn-headed worm larvae were ever found in control grubs selected at random from each of the various lots of grubs used.

The procedure employed in testing the viability of the eggs was as follows: Definite quantities (by volume) of eggs were thoroughly mixed with 30 cc. of slightly moist soil that had first been sifted through a 30-mesh-to-the-inch screen and then autoclaved for 30

[1] Received for publication July 15, 1941.

[2] SCHWARTZ, BENJAMIN. INTERNAL PARASITES OF SWINE. U. S. Dept. Agr. Farmers' Bul. 1787, 46 pp., illus. 1937.

[3] Van Cleave [4] has designated this stage of the parasite as a "shelled embryo." However, as a matter of convenience the term "egg" is used throughout this paper.

[4] VAN CLEAVE, H. J. DEVELOPMENTAL STAGES IN ACANTHOCEPHALAN LIFE HISTORIES. Lenin Acad. Agr. Sci. K. J. Skrjabin Commemoratory Vol., pp. 739–743. 1937.

minutes. The mixtures of soil and eggs were placed in 50-cc. metal screw-top bottles that had been sterilized in an autoclave. One grub was added to each culture and kept for 10 days at a temperature of 21° to 26° C. At the end of the 10-day period each grub was decapitated, the intestine dissected out, opened lengthwise, and then washed gently in water to remove soil particles. The intestinal tissue was then pressed between glass slides and examined microscopically for the presence of young thorn-headed worm larvae, or acanthors.[5]

In tests of the effect on eggs of high temperatures instantaneously applied, 0.5-cc. quantities of eggs were subjected to temperatures ranging from 45° to 100° C. at intervals of 5°, as follows: The eggs were pipetted into beakers each containing 100 cc. of water that had been heated to the desired temperature by means of a water bath. Immediately thereafter, the beakers were removed from the water bath and 250 cc. of ice water added to reduce rapidly the temperature of the water surrounding the eggs to approximately 30° or lower. The eggs were then concentrated by sedimentation, mixed with soil, and their viability determined by grub-feeding tests.

Another series of tests involved eggs exposed for 10 minutes to each of a series of temperatures ranging from 36° to 90° C. at intervals of 5°. Eggs in 0.5-cc. quantities were pipetted into bottles containing 10 cc. of water at the desired temperature. The bottles were then tightly stoppered and immersed in a water bath maintained at the same temperature. At the expiration of 10 minutes the bottles were removed from the bath, the stoppers drawn, the supernatant fluid quickly decanted, and the bottles filled with ice water. The viability of the eggs was then ascertained by grub-feeding tests. For both series of tests control cultures were made from 0.5-cc. quantities of eggs that had been subjected to a temperature of 26° C.

In tests involving continued freezing, 0.5-cc. quantities of eggs in a series of tightly corked sputum jars of water were frozen at temperatures of −10° to −16° C. in the freezing compartment of a mechanical refrigerator. At the same time, similar quantities of eggs that had been spread in thin layers on glass plates and then dried 10 minutes in a current of air were refrigerated to test the effect on egg viability of combined desiccation and freezing. Control preparations of eggs in water were maintained at temperatures of 21° to 26°. At intervals, as shown in table 3, preparations of eggs in water and of those in the dried condition were removed from the refrigerator, allowed to thaw at room temperature, and mixed with soil for grub-feeding tests.

In tests on the effect of continuous drying, 1 cc. of egg concentrate was spread evenly in a thin layer on the surface of each of a series of 4-inch-square glass plates and dried for approximately 10 minutes in a current of air at room temperature (26° C.). Some of the preparations were kept at temperatures of 5° to 9°, others at 21° to 26°, and still others at 37° to 39° C. At intervals, as shown in table 4, eggs from one of the preparations from each temperature group were scraped from the glass plate, mixed with moist sterile soil, and the

[5] Van Cleave [4] first used the term "acanthor" to designate both the thorn-headed worm larvae contained in eggs and the first-stage larvae in the intermediate host. As a matter of convenience the term is used throughout this paper.

number of viable eggs present in each sample estimated by grub-feeding tests.

To test the effect of alternate wetting and drying, 0.5-cc. quantities of eggs were scattered over the surface of a layer of autoclaved soil in each of a series of uncovered Petri dishes. Some of the preparations were maintained at temperatures of 2° to 5° C. and others at 37° to 39°. The preparations were kept under observation for 551 days, and during this time those at the lower temperatures were moistened five times and those at the higher temperatures nine times at irregular intervals of 30 to 150 days. The viability of the eggs was then tested by grub feeding.

In tests on the effect of ultraviolet irradiation, eggs dried on glass plates and eggs in Syracuse watch glasses in 10 cc. of water were subjected to irradiation from a quartz mercury-vapor arc lamp. Some of the dried preparations were one egg in thickness; others were several eggs thick. The cultures were irradiated at a distance of 18 inches from the tube. During irradiation the air temperature at the level of the cultures was maintained at or below 36° C. by means of a current of air. Control cultures consisting of eggs in a dried condition and in shallow water were kept at temperatures of 37° to 39° during the longest period that the test eggs were subjected to irradiation, and were not exposed to ultraviolet irradiation at any time.

EXPERIMENTAL RESULTS

EFFECT OF HIGH TEMPERATURES

In a preliminary series of 13 tests, in which the eggs were subjected to temperatures ranging from 45° to 100° C., a few apparently survived instantaneous exposure to temperatures as high as 100°, as judged by results of grub-feeding tests (table 1, tests 1 to 13). These results were not borne out in a subsequent series of 7 tests (table 1, tests 14 to 19), in which all equipment was autoclaved before being used. In

TABLE 1.—*Effect on thorn-headed worm eggs of high temperatures instantaneously applied*

SERIES 1.—ALL EQUIPMENT NOT AUTOCLAVED BEFORE USE

Test No.	Temperature of water	Grubs examined	Average number of acanthors recovered per grub	Test No.	Temperature of water	Grubs examined	Average number of acanthors recovered per grub
	°C.	Number			°C.	Number	
1	45	3	1,609	8	80	3	53
2	50	3	1,395	9	85	2	2
3	55	3	1,248	10	90	3	5
4	60	4	474	11	95	3	3
5	65	3	1,211	12	100	3	6
6	70	3	89	13	100	3	0
7	75	3	174				

SERIES 2.—ALL EQUIPMENT AUTOCLAVED BEFORE USE

14	50	3	1,169	18	70	6	0
15	55	4	320	19	75	3	0
16	60	4	398	20 [1]	26	1	1,544
17	65	4	3				

[1] Control.

the latter series of tests, no eggs survived instantaneous exposure to 70° or higher. It is concluded, therefore, that the apparent survival of some eggs at temperatures higher than 70° in the first series may have been due to contamination of the cultures. This may have occurred as a result of small numbers of viable eggs adhering to the dishes, pipettes, spatulas, or other equipment used in culturing the eggs after their exposure to heat.

In these tests it was observed that the majority of the eggs were unaffected, apparently, by a temperature of 45° C. This is shown by the fact that grubs fed eggs that had been subjected to this temperature harbored an average of 1,609 acanthors in the first series of tests and 1,169 in the second series, as compared with 1,544 in the control grub. In tests at higher temperatures, however, the numbers of eggs that survived decreased gradually as the temperature was raised. In the second series of tests only 3 acanthors, on the average, were recovered from the test grubs fed eggs exposed to 65° C. In this series, as already stated, no eggs survived a temperature of 70°.

In a carefully controlled series of 12 tests (table 2), in which eggs were exposed 10 minutes to temperatures ranging from 36° to 90° C., none survived at 60° or higher, except as indicated in the footnote of table 2.

TABLE 2.—*Effect on thorn-headed worm eggs of high temperatures sustained 10 minutes*

Test No.	Temperature of water	Grubs examined	Average number of acanthors recovered per grub	Test No.	Temperature of water	Grubs examined	Average number of acanthors recovered per grub
	°C.	Number			°C.	Number	
1	36	1	1,164	8	70	3	0
2	40	3	1,116	9	75	3	0
3	45	3	597	10	80	3	[1] 0.3
4	50	3	582	11	85	3	0
5	55	3	1,131	12	90	3	0
6	60	3	0	13 [2]	26	4	720
7	65	3	0				

[1] 1 acanthor was found in 1 grub in this test and was definitely abnormal.
[2] Controls.

EFFECT OF CONTINUOUS FREEZING IN WATER AND IN A DRIED CONDITION

As shown in table 3, continuous exposure to temperatures varying from −10° to −16° C. for periods as long as 140 days failed to destroy the viability of the majority of eggs, regardless of whether they were frozen in water or in a dried condition. With the exception of tests 7 and 8 and 11 and 12, freezing in water appeared to be somewhat more effective in reducing egg viability than freezing in a dried condition. In tests 9 to 12, the number of acanthors recovered from grubs fed eggs at intervals during this period was comparable to the number recovered from corresponding control grubs. The smaller number of acanthors recovered from tests 1 to 8 was probably due to a difference in activity of the grubs used.

TABLE 3.—*Effect on thorn-headed worm eggs of freezing in water and in a dried condition* [1]

Test No.	Condition of eggs at time of freezing	Period of exposure to freezing (−10° to −16° C.)	Grubs examined	Average number of acanthors recovered per grub	Test No.	Condition of eggs at time of freezing	Period of exposure to freezing (−10° to −16° C.)	Grubs examined	Average number of acanthors recovered per grub
		Days	*Number*				*Days*	*Number*	
1	In water	4	2	173	8	Dried	42	1	140
2	Dried	4	2	284	9	In water	81	1	796
3	In water	11	2	310	10	Dried	81	1	1,496
4	Dried	11	1	562	11	In water	140	1	902
5	In water	24	2	322	12	Dried	140	1	721
6	Dried	24	2	459	13 [2]		0	5	810
7	In water	42	1	164					

[1] The dried eggs, before being frozen, had been spread in thin layers on glass plates and exposed to air for 10 minutes.
[2] Controls in water at 21° to 26° C.

EFFECT OF CONTINUOUS DRYING

The data presented in table 4 show that continuous drying at temperatures of 21° to 26° C. for 265 days and at 5° to 9° and 37° to 39° for 50 days failed to destroy the viability of the eggs. Data already presented in table 3 show that the majority of eggs exposed in a dried condition to temperatures ranging from −10° to −16° survived for as long as 140 days. These findings indicate that continuous drying, which is effective in destroying the viability of eggs and larvae of some parasites, is comparatively ineffective in destroying the viability of eggs of the swine thorn-headed worm.

In tests 3 to 6, the numbers of acanthors recovered from the grubs were noticeably smaller than those in the remainder of the tests. This difference was due to the fact that the temperatures at which the grubs were kept were lowered to 7° to 15° C., because of an unforseen reduction in laboratory temperature. Under these conditions the grubs became partially inactive, did not feed readily, and, therefore, may not have ingested so many eggs as those kept at higher temperatures.

TABLE 4.—*Effect of continuous drying on thorn-headed worm eggs*

Test No.	Period of exposure to drying	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub	Test No.	Period of exposure to drying	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub
	Days	*°C.*	*Number*			*Days*	*°C.*	*Number*	
1	16	5–9	1	1,778	8	35	21–26	1	2,789
2	16	21–26	2	1,467	9	35	37–39	1	1,788
3	16	37–39	2	817	10	50	5–9	2	1,354
4	25	5–9	1	550	11	50	21–26	2	1,111
5	25	21–26	1	394	12	50	37–39	2	1,507
6	25	37–39	1	346	13	265	21–26	1	1,027
7	35	5–9	1	1,064	14 [1]	0		5	1,453

[1] Controls.

EFFECT OF ALTERNATE WETTING AND DRYING

The data presented in table 5 show that the viability of practically all eggs on soil subjected to alternate wetting and drying at temperatures of 37° to 39° C. were destroyed in 368 days and that all eggs were destroyed in 551 days. At temperatures of 2° to 5°, however, comparatively large numbers of acanthors were recovered at the end of these periods. Manter [6] and others have observed that when eggs are dried and then placed in water some of the shells break, thereby allowing the acanthors to escape. This observation has also been made by the writer and may explain the loss of egg viability in tests in which the samples were subjected to repeated wetting and drying at 37° to 39° (tests 2, 4, 6, and 8 in table 5). The fact that at 2° to 5° the soil of the samples did not become so thoroughly dry as that at 37° to 39° accounts for the greater survival of eggs under these conditions.

TABLE 5.—*Effect of alternate wetting and drying on thorn-headed worm eggs*

Test No.	Period of exposure	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub	Test No.	Period of exposure	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub
	Days	*°C.*	*Number*			*Days*	*°C.*	*Number*	
1	102	2–5	1	2,852	6	368	37–39	1	1
2	102	37–39	1	276	7	551	2–5	2	1,194
3	132	2–5	1	727	8	551	37–39	2	0
4	132	37–39	1	352	9 [1]	551	2–5	1	270
5	368	2–5	1	272					

[1] Control continuously dry.

EFFECT OF ULTRAVIOLET IRRADIATION

Table 6 summarizes the data on the effect of ultraviolet irradiation on eggs of the thorn-headed worm. Tests 1 to 11 show the effect of the irradiation on dry eggs spread in several layers on glass plates. In all cases, as compared with the controls, there was some reduction in the number of acanthors recovered, although exposures up to 40 minutes failed to destroy viability in all the eggs. When eggs were spread on glass plates in a single layer by using smaller quantities of egg concentrate and then exposed to irradiation (tests 12 to 32), only an insignificant number survived a 10-minute exposure. This finding indicates that direct exposure to strong ultraviolet irradiation will render the eggs of this parasite nonviable very quickly. Eggs placed in shallow water and then irradiated (tests 33 to 51) showed a tendency to decrease gradually in viability as the period of irradiation was lengthened. Some eggs, however, survived irradiation for periods of 120 minutes (test 50).

[6] MANTER, H. W. NOTES ON THE EGGS AND LARVAE OF THE THORNY-HEADED WORM OF HOGS. Amer. Micros. Soc. Trans. 47: 342–347, illus. 1928.

TABLE 6.—*Effect of ultraviolet irradiation*

Test No.	Quantity of egg concentrate	Period of exposure	Grubs examined	Average number of acanthors recovered per grub
	Cc.	*Minutes*	*Number*	
EGGS DRIED IN SEVERAL LAYERS ON GLASS PLATES				
1	1	30	2	1,077
2		40	2	267
3[1]		0	2	1,770
4	0.5	5	2	342
5		10	2	144
6		15	2	122
7		20	2	41
8		25	2	129
9		30	2	89
10		35	2	118
11[1]		0	2	419
EGGS DRIED IN SINGLE LAYER ON GLASS PLATES				
12	0.2	10	2	1
13		20	2	0
14–23		[2] 30–120	12	0
24[1]	0.01	0	2	1,097
25		10	2	3
26		20	2	1
27		30	2	0
28		40	2	2
29		50	2	0
30		60	4	0
31		70	4	0
32[1]		0	2	262
EGGS IN WATER				
33	0.05	10	2	237
34		20	2	15
35		30	1	6
36		40	2	0
37		50	2	5
38[1]		0	1	383
39	0.2	10	1	882
40		20	1	1,106
41		30	1	528
42		40	1	831
43		50	1	516
44		60	1	79
45		70	1	1,342
46		80	2	101
47		90	2	2
48		100	1	88
49		110	1	21
50		120	1	208
51[1]		0	2	1,042

[1] Controls.
[2] 10-minute intervals.

SUMMARY AND CONCLUSIONS

Tests under laboratory conditions were carried out on the effects of high and low temperatures, drying, alternate wetting and drying, and ultraviolet irradiation on the viability of eggs of the swine thorn-headed worm (*Macracanthorhynchus hirudinaceus*). The criterion used to test the viability of the eggs was the ability of the contained larvae, or acanthors, to infect grubs of the green June beetle (*Cotinis nitida* (L.)). The work was carried on in 1938 and 1939 at the

United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Eggs in water were destroyed by instantaneous exposure to a temperature of 70° C. and by 10 minutes' exposure to a temperature of 60°. In one series of preliminary tests, not so well controlled, a small number of eggs survived higher temperatures.

The majority of eggs in water and in a dried condition survived continuous exposure to temperatures ranging from −10° to −16° C. for as long as 140 days, when the test was terminated.

No appreciable reduction in number of viable eggs was observed in dry preparations exposed for 50 days at temperatures of 5° to 9° C. and 37° to 39°, and for 265 days at temperatures of 21° to 26°.

Alternate wetting and drying at temperatures of 37° to 39° C. destroyed the viability of eggs on soil in 368 days. On the other hand, eggs subjected to alternate wetting and drying at temperatures of 2° to 5° were still viable after 551 days.

Ultraviolet irradiation applied at a distance of 18 inches destroyed nearly all the dry eggs in a single layer in 10 minutes. Some eggs in films several eggs thick survived for 40 minutes. Some eggs in shallow water survived irradiation for as long as 120 minutes.

Data presented in this paper on the resistance, to experimental conditions, of eggs of thorn-headed worms furnish, in part, an explanation for the fact that eggs of this parasite have remained viable on soil for as long as 3½ years under conditions at Beltsville, Md., as reported in previous work.[7]

[7] SPINDLER, L. A., and KATES, K. C. SURVIVAL ON SOIL OF EGGS OF THE SWINE THORN-HEADED WORM, MACRACANTHORHYNCHUS HIRUDINACEUS. (Abstract) Jour. Parasitol. 26 (6) Sup. : 19. 1940.

INHERITANCE OF SMOOTH AND PITTED BOLLS IN PIMA COTTON [1]

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INTRODUCTION

In cotton relatively few characters segregate in simple Mendelian fashion. Kearney [2] lists only 14 allelic pairs of characters. Additions have been made since his list was compiled, but the number is still small. Conspicuous allelic characters, if not deleterious, are of interest to cotton breeders for the reason that they can be transferred from one variety to another and utilized as a mark of identification. The discovery of a character of this nature, a smooth-boll variation in Pima cotton, is reported in this paper.

OBSERVATIONS AND EXPERIMENTAL WORK

The boll surface in Pima, an American-Egyptian variety of *Gossypium barbadense* L., is dotted with craterlike pits (fig. 1, *A*). A large

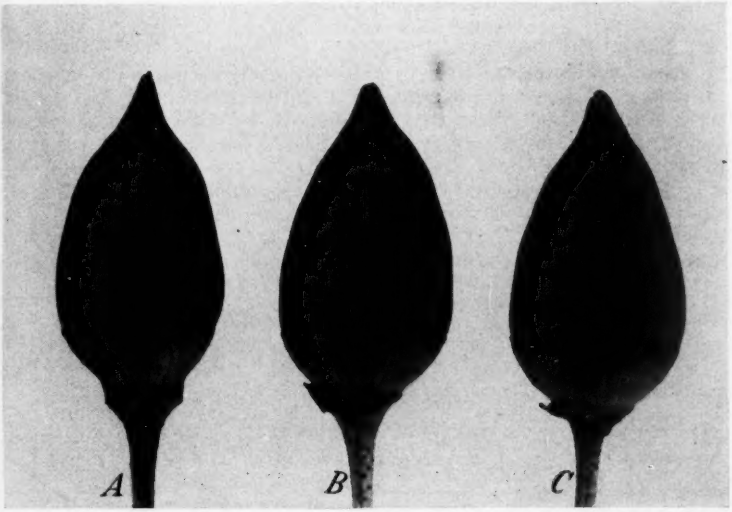

FIGURE 1.—Pitted, intermediate, and smooth cotton bolls: *A*, Normal Pima, pitted; *B*, Pima × P Hope F_1, showing intermediate pitting; *C*, P Hope, smooth. × about 1¼ (bolls approximately 40 mm. long).

oil or resin gland, which bleeds freely when bruised, is embedded at the base of each pit. In the variant smooth-boll form the bolls appear to be smooth (fig. 1, *C*), the pits being very obscure. Also, the glands are smaller than in typical Pima and do not bleed so freely when the surface of the boll is bruised.

[1] Received for publication June 7, 1941.

[2] KEARNEY, THOMAS H. GENETICS OF COTTON. Jour. Hered. 21: 325-336, illus. 1930. See p. 331.

The parent of the smooth-boll strain was found in 1932, by Claude Hope,[3] in a field of Pima cotton that was grown for increase of pure planting seed at the United States Field Station, Sacaton, Ariz. A few hybrids between *Gossypium barbadense* and *G. hirsutum* L. appear at Sacaton every year in the fields that are used for increasing seed, so there is a possibility that the parent of the smooth-boll strain was a dilute hybrid of this origin. However, for convenience the strain is treated herein as *G. barbadense*, as the origin of the parent plant will always remain conjectural. The plant was much like Pima, except for the conspicuously different character of the boll surface.

Only open-pollinated seeds were obtained in 1932, there having been no opportunity to protect the flowers against cross-pollination. Nevertheless, in the following year all of the progeny produced smooth bolls. The strain, which was designated "P Hope," has bred true for eight generations.

In 1933 the smooth-boll strain was crossed with normal Pima. The surface of the bolls proved to be intermediate in degree of pitting in all individuals of the first generation. In subsequent years, second-generation and third-generation populations were grown, as well as several backcrosses. The classifications of the several populations are given in table 1.

Segregation in F_2, and in F_3 of an intermediate F_2 individual, approximated a 1 : 2 : 1 ratio, and segregation in backcrosses of F_1 individuals with individuals of the pitted and smooth parental families, respectively, approximated a 1 : 1 ratio. The departure of the observed distribution from the distribution calculated on the assumption of a single-factor difference is in no instance significant. It seems clear, therefore, that smooth-boll inheritance is monohybrid in this intraspecies cross.

TABLE 1.—*Classification of the F_1, F_2, F_3, and backcross populations in a cotton cross involving smooth and pitted boll surface* [1]

Combination	Year	Classification of parent	Number of plants	Observed distribution			χ^2	P
				Smooth	Intermediate	Pitted		
First crosses:								
Smooth × pitted F_1	1934		29	0	29	0		
Smooth × pitted F_2	1935	Intermediate	50	11	28	11		
Do	1938	do	68	13	36	19		
Do	1940	do	53	12	32	9		
Combined F_2 populations			171	36	96	39	2.68	0.3
Smooth × pitted F_3	1936	Smooth	9	9	0	0		
Do	1939	do	41	41	0	0		
Do	1936	Pitted	10	0	0	10		
Do	1936	Intermediate	26	6	14	6	.15	.9
Backcrosses:								
Intermediate × pitted F_1	1936		29	0	12	17		
Do	1937		34	0	14	20		
Combined populations			63	0	26	37	1.92	.2
Intermediate × smooth F_1	1936		28	10	18	0		
Do	1937		34	21	13	0		
Combined populations			62	31	31	0		

[1] The values for χ^2 of the departures from a 1 : 2 : 1 and a 1 : 1 ratio, respectively, are computed on the assumption of a single-factor difference. For formula see FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 pp., illus. 1928.

[3] Then a member of the staff of the Division of Cotton, Rubber, and Other Tropical Plants (now the Division of Cotton and Other Fiber Crops and Diseases), Bureau of Plant Industry.

DISCUSSION

Although the presence or absence of pits on the bolls is of taxonomic importance in the genus *Gossypium*, the inheritance had not been analyzed successfully previous to the present study, according to Kearney [4] and Harland [5] (*pp. 117–118*). Harland (*p. 58*) suggests that Mendelian segregation may be obscured in interspecific cotton crosses by the "large number of different genetical backgrounds upon which the given pair of alleles would manifest themselves." This hypothesis offers an explanation for the difficulty encountered by investigators who have worked with interspecific hybrid populations. The present success with the pitted-boll character may be accounted for on the assumption of similar genetic backgrounds in P Hope and ordinary Pima. Even though P Hope may have originated by natural hybridization between Pima (*G. barbadense*) and upland cotton (*G. hirsutum*), a Pimalike genetic background could have been acquired by repeated natural backcrossing on Pima prior to the discovery of the parent P Hope plant.

The symbols B^p and B^s, respectively, are proposed for the allelic boll surface characters pitted and smooth.

[4] KEARNEY, THOMAS H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Dept. Bul. 1164, 57 pp., illus. 1923.

[5] HARLAND, SYDNEY CROSS. THE GENETICS OF COTTON. 193 pp., illus. London. 1939.

INHERITANCE OF CLUSTER HABIT AND ITS LINKAGE RELATION WITH ANTHOCYANIN PIGMENTATION IN UPLAND COTTON[1]

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INTRODUCTION

Future progress in the improvement of the cotton plant by breeding is dependent to some extent upon a more complete genetic analysis of the various wild and cultivated species. The accumulation of knowledge regarding the mode of inheritance of characters, the identification of these characters with the chromosomes, and the mapping of the chromosomes will increase the certainty and rapidity of cotton improvement.

Genetic studies in New World cottons have resulted in definite information regarding the mode of inheritance of forty-odd factors that affect 14 characteristics. Among these are the genes that affect the anthocyanin pigmentation and cluster habit.

The presence of anthocyanin results in a red or wine-red coloration in cotton plants. Some degree and type of anthocyanic pigmentation occurs in practically every species of the genus *Gossypium*. In *G. hirsutum*, according to Ware's (*18*)[2] description, all exposed parts of plants homozygous for the characteristic possess a deep red color. In the heterozygous condition the color is a dilute red, and the anthocyanin pigment is somewhat restricted to the plant stems, the petioles and veins of the leaves, and the portion of the corolla exposed to sunlight; while the red coloration of the leaf lamina is very dilute.

McLendon (*12*), Thadani (*17*), Ware (*18*), Carver (*1*), and Harland (*4*) have shown that anthocyanin pigmentation is controlled by a single genetic-factor pair. Ware (*19, 20*) found that the factors for red plant and "okra leaf," for red plant and green lint, and for red plant and brown lint segregate independently. Killough and Horlacher (*11*) reported an independent assortment for the genes affecting the red-plant and virescent-yellow characteristics.

Certain strains of upland cotton are characterized by an excessive shortening of the fruiting branches, and the bolls are borne in clusters. Thadani (*17*) found that an intra-upland cross between cluster and noncluster types resulted in a normal F_1 and a segregation of 3 normal to 1 cluster in the F_2 generation, indicating that a single factor pair was involved. Texas workers (*16, p. 54*) found a cluster type in the Durango variety and reported that when crossed with a noncluster type it behaved as a recessive. Harland (*6*) found that cluster in some crosses behaved in a complicated manner, showing all grades of blending between cluster and normal in the F_2 segregation, and con-

[1] Received for publication March 19, 1941. Cooperative investigations of the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 117.

cluded that the cluster habit is determined by one basic gene and that modifiers may determine minor differences in the degree to which cluster is expressed.

In summarizing the heritable relationships of characteristics in New World cottons, Harland (*6*) indicated that the genes affecting virescent-yellow and cluster habit are inherited independently.

Only two definite cases of linkage in the New World cottons have been reported. However, the heritable relationships of only 56 pairs of genes have been recorded; and since there are 26 (haploid) chromosomes, it is not surprising that more cases of linkage have not been established.

Thadani (*17*) studied the heritable relationships of anthocyanin pigmentation and cluster habit in the F_2 generation of a red plant, cluster habit $\times$ green plant, noncluster habit cross, and found linkage. The calculation of cross-over values from Thadani's data is not easily accomplished, since the monohybrid ratios were rather disturbed and since no green-cluster recombination phenotypes were obtained in the segregation. Harland (*5*), who studied the linkage relationship in the backcross segregation, found that the anthocyanin and cluster genes belong to the same linkage group and obtained a cross-over value of 13.9 percent.

Harland (*5*) has reported another case of linkage in New World cottons. He found in a small backcross population that crinkled and green lint were linked with 5 percent crossing over. Three clear cases of linkage in Asiatic cottons have been reported. Hutchinson (*7*) found that the genes for leaf shape and brown lint were linked and that 29.9 percent cross-overs were obtained. Yu (*21*) has reported that yellow seedling and anthocyanin pigmentation are linked and that the cross-over value is about 9 percent. Yu (*22*) also found that curly leaf and virescent bud in Asiatic cotton are linked, with a cross-over value not far from 16.6 percent.

In the cotton genetic studies now being conducted by the Bureau of Plant Industry, in cooperation with the Mississippi Agricultural Experiment Station, the heritable relations of a large number of characteristics are being determined. Since the mode of inheritance of the cluster habit of strains isolated from certain varieties has not been reported, and since, in order to be of predictive value, recombination fractions should be based upon the results of several repetitions, the studies regarding the mode of inheritance of the cluster-habit character and the chromosomal relationships of cluster habit and red plant have been repeated. The results presented here are not considered conclusive, nor are they presented to refute the work of earlier investigators. For the most part, the findings of these geneticists have been verified, and when divergent conclusions are expressed, they are offered mainly for widening the scope of investigation and as a stimulation to further studies regarding these genetic phenomena.

MATERIALS AND METHODS

Two strains of *Gossypium hirsutum* L. were used in these studies. The Winesap, anthocyanin-pigmented type (fig. 1, *A*), was obtained from the Arkansas Agricultural Experiment Station and had been inbred for eight generations. The Bawaka, cluster-habit type (fig. 1, *B*), was obtained through the Division of Plant Exploration and Intro-

duction of the Bureau of Plant Industry from the Turkestan Plant Breeding Station, Tashkent, U. S. S. R., and, no doubt, is a derivative of an introduced American upland variety. The phenotypically red, noncluster strain has been designated genotypically as $R_1^{RO}R_1^{RO}ClCl$, and the phenotypically green, cluster strain has been designated genotypically as $r_1^{ro}r_1^{ro}clcl$, according to the gene symbols proposed by Hutchinson and Silow (*8*). The superscript indicates that the effect of the R_1 gene does not extend to the petal spot, as does the effect of certain duplicate anthocyanin genes.

The methods of selecting parent plants and of self- and cross-pollination were the same as described by Ware (*18*). Crosses were made in 1936 and 1937 between the Winesap and Bawaka lines. The Winesap was used as the female parent in all crosses, since emasculation is more difficult and shedding is greater in the Bawaka strain. Progenies were grown in 25-foot rows, each containing 20 to 25 hills. In those progenies that segregated for anthocyanin pigmentation the proportion of red, intermediate red, and green plants was verified, and the progenies were then thinned to a 1 : 2 : 1 ratio in the F_2 and F_3 populations and to a 1 : 1 ratio in the backcross population. This method of planting and thinning was necessary, since singly spaced seed have difficulty in germinating because of the compact surface of the soil and thickly spaced plants do not develop sufficiently for accurate classification of the cluster habit.

Upon maturity of the plants the frequencies of each of the following phenotypes in the F_2 and F_3 generations were determined: Red noncluster, intermediate-red noncluster, green noncluster, red cluster, intermediate-red cluster, and green cluster. The backcross segregates were classified into four phenotypic classes: Intermediate-red noncluster, intermediate-red cluster, green noncluster, and green cluster. To facilitate linkage calculations, the intermediate-red noncluster segregates were placed in the red noncluster phenotypic class, and the intermediate-red cluster segregates were placed in the red cluster phenotypic class. The F_2 segregates were divided into four families and the F_3 segregates into three families on the basis of different individual plants that were used in the parental combinations. However, there is no obvious reason for adopting this grouping, since all male and all female parents were from the same respective inbred line. Both F_2 and F_3 progenies of double heterozygous plants were used in determining the linkage values. One family of backcross segregates was propagated and analyzed.

INHERITANCE OF CLUSTER HABIT

F_1 POPULATIONS

The F_1 generations of the noncluster × cluster cross, grown in 1937 and 1938, were composed of 12 and 16 plants, respectively. These plants (fig. 1, *C*) were classified as noncluster, since there was no obvious shortening of the sympodial branches. However, there appeared to be a rather consistent difference between these F_1 plants and the noncluster parental line. In the axis of the branch and the regular flower pedicel of the F_1 plants, an axillary fruiting bud formed. These "squares" were shed before they developed into flowers; and when the plant was mature, it was morphologically similar to the noncluster parent.

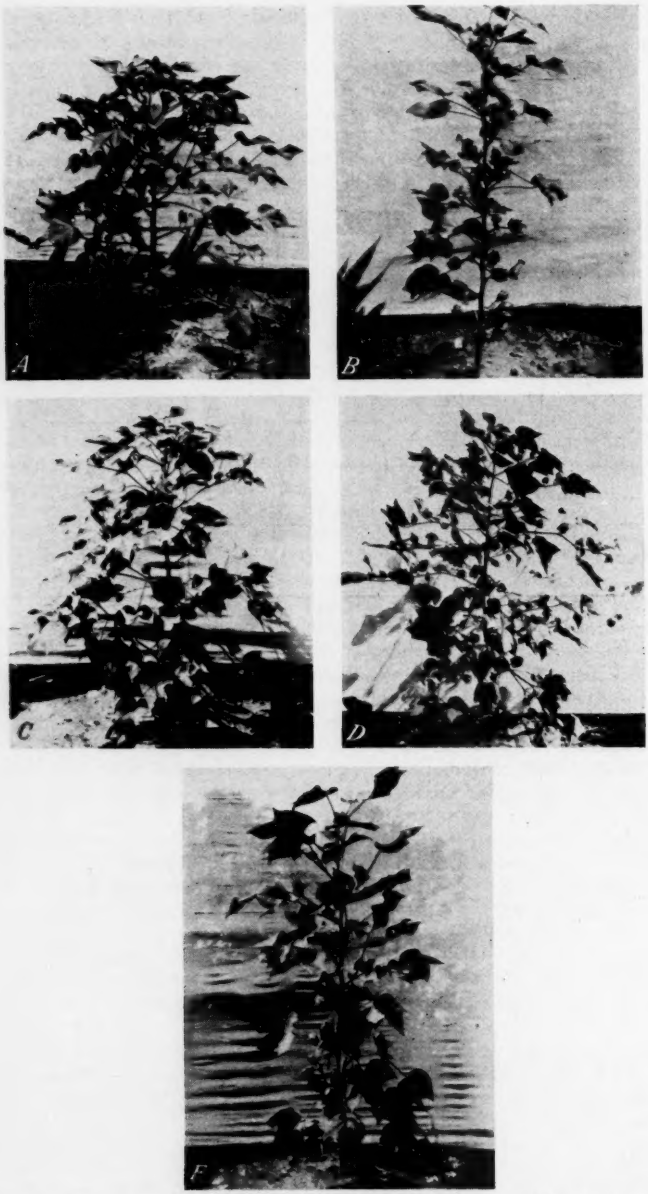

FIGURE 1.— Parents and F_1 and F_2 of hybrid upland cotton: *A*, Noncluster parent R_1^{RO}; *B*, cluster parent r_1^{ro}; *C*, F_1 of cross between *A* and *B* is noncluster; F_2 progeny segregates into three noncluster (*D*) and one cluster (*E*).

F_2 POPULATIONS

The F_1 plants were self-pollinated; in 1938 and 1939, 672 F_2 plants were grown to maturity. These progenies segregated into two distinct types, noncluster and cluster (fig. 1, *D* and *E*), respectively. In table 1 the phenotypic-class frequencies of the F_2 generation of the four families, the test for goodness of fit with the expected 3 : 1 ratio, and the test for agreement between families are given. The component of χ^2 attributed to the heterogeneity between families was obtained by subtracting the χ^2 for deviation from the sum of the χ^2's attributed to the four families, according to the procedure suggested by Mather (*13*). The observed and calculated frequencies agree quite closely; the probability value, according to Fisher's (*2*) table of χ^2 being 0.61. The segregations within the families agree with one another, the probability value being 0.57. These two agreements indicate that the cluster and noncluster characteristics differ by one genetic-factor pair.

TABLE 1.—*Phenotypic-class frequencies of the F_2 generation of the noncluster × cluster cross; the test for goodness of fit with the expected ratio; and the test for agreement between families*

Family	*Cl*	*cl*	Total	χ^2	Degrees of freedom	Probability
1	16	7	23	.3623	1	
2	62	27	89	1.3520	1	
3	100	38	138	.4734	1	
4	320	102	422	.1548	1	
Total	498	174	672	2.3425	4	
Calculated (3 : 1)	504	168	672			
Deviation				.2857	1	.61
Heterogeneity				2.0568	3	.57

BACKCROSS POPULATION

In table 2 the frequencies of the *Cl* and *cl* phenotypes of the backcross ($r_1^{ro}cl/R_1^{RO}Cl \times r_1^{ro}cl/r_1^{ro}cl$), and the test for goodness of fit with the expected 1 : 1 ratio are given. The observed and calculated frequencies agree satisfactorily, the probability value being 0.50.

TABLE 2.—*Phenotypic-class frequencies of the backcross ($r_1^{ro}cl/R_1^{RO}Cl \times r_1^{ro}cl/r_1^{ro}cl$) and the test for goodness of fit with the expected ratio*

Phenotypic class	Observed	Calculated (1:1)	χ^2	Degrees of freedom	Probability
Cl	66	70			
cl	74	70			
Total	140	140	.457	1	.50

DETECTION OF LINKAGE

F_2 AND F_3 POPULATIONS

The frequencies of the four phenotypic classes of the F_2 generation of families 1, 2, 3, and 4, and of the F_3 segregation of the F_2 double heterozygotes of families 1, 2, and 3, were used in the detection of linkage.[3] The χ^2 for the segregation of these double heterozygous

[3] While the frequencies of some F_3 lines were somewhat smaller than was desirable, the lines were tested to determine whether they came from coupling or repulsion recombination, as suggested by Immer (*10*). None of the F_3 lines used in these studies conformed satisfactorily to the repulsion-recombination hypothesis.

($R_1^{RO}r_1^{ro}Clcl$) plants includes three components: (1) Deviation of the R_1^{RO}, r_1^{ro} segregation from its 3 : 1 expectation, (2) deviation of the *Cl*, *cl* segregation from the single-factor expectation, and (3) the deviation of the joint segregation from its expectation of independent inheritance. The following formulas, which are discussed by Mather (*13*), were used in calculating the three χ^2 values:

$$\chi^2 R_1^{RO} = (R_1^{RO}Cl + R_1^{RO}cl - 3r_1^{ro}Cl - 3r_1^{ro}cl)^2/3n$$

$$\chi^2 Cl = (R_1^{RO}Cl - 3R_1^{RO}cl + r_1^{ro}Cl - 3r_1^{ro}cl)^2/3n$$

$$\chi^2 \text{ linkage} = (R_1^{RO}Cl - 3R_1^{RO}cl - 3r_1^{ro}Cl + 9r_1^{ro}cl)^2/9n$$

The first two components, corresponding to single-factor ratios, are based upon the formula for calculating the χ^2 values for the 3 : 1 segregation; the third component follows from orthogonality.

The goodness of fit tests were made with two groupings of the phenotypic frequencies, the family grouping and the generation grouping. In each analysis the χ^2 was divided into two parts: (1) That concerned with the deviation of all groups taken together from the expected ratio, and (2) that concerned with the lack of agreement between the groups.

The phenotypic-class frequencies of the combined F_2 and F_3 progenies, the tests for goodness of fit for the single-factor and joint segregations for each family and for the combined populations, and the tests for agreement between families are given in table 3. The thinned population of red and green phenotypes corresponds closely to the expected 3 : 1 ratio, the probability value being 0.44. The segregation into *Cl* and *cl* phenotypic classes conformed satisfactorily to the single-factor hypothesis, the probability value being 0.67. In each case the heterogeneity between families was not significant, the probability values for R_1^{RO} and *Cl* being 0.74 and 0.61, respectively. The χ^2 for linkage, 324.3630, corresponds to a very small probability value, and it is thus quite obvious that the single-factor ratios account for very little of the total χ^2 but that there is a large component corresponding to linkage. The heterogeneity χ^2 for linkage was not significant, the probability value being 0.42. Thus the four families agree in showing linkage of the R_1^{RO} and *Cl* factors.

TABLE 3.—*Phenotypic-class frequencies of combined F_2 and F_3 progenies of the cross red noncluster × green cluster; the tests for goodness of fit for the single-factor and joint segregations for each family and for the combined populations; and the tests for agreement between families*

Family	Frequencies				Degrees of freedom	$R_1^{RO}r_1^{ro}$ segregation		*Clcl* segregation		Joint segregation	
	$R_1^{RO}Cl$	$R_1^{RO}cl$	$r_1^{ro}Cl$	$r_1^{ro}cl$		χ^2	Probability	χ^2	Probability	χ^2	Probability
1	80	9	12	19	1	.04444	.....	.1778	.....	32.7259	.....
2	175	30	24	48	1	.1456	.....	1.4741	.....	79.4324	.....
3	122	20	14	29	1	.3045	.....	.2180	.....	47.4240	.....
4	282	24	38	78	1	1.3934	.....	.1548	.....	167.6682	.....
Total	659	83	88	174	4	1.8879	.....	2.0247	.....	327.2505	.....
Calculated 9 : 3 : 3 : 1	564.75	188.25	188.25	62.75	.....	.....	.....	.....	.....	.....	.....
Deviation	.....	.....	.....	.....	1	.6428	.44	.1912	.67	324.3630	.00
Heterogeneity	.....	.....	.....	.....	3	1.2451	.74	1.8355	.61	2.8875	.42

In table 4 the phenotypic-class frequencies of the combined four families of F_2 progenies and combined three families of F_3 progenies, the tests for goodness of fit for the single-factor and joint segregations for each generation and for the combined populations, and the tests for agreement between generations are given. The χ^2 and probability values for deviation for each of the single-factor segregations and for the joint segregation are the same as given in table 3. For none of the three segregations is the heterogeneity χ^2 significant, and it is shown that the two generations are homogeneous for each component of the total χ^2.

TABLE 4.—*Phenotypic-class frequencies of combined four families of F_2 progenies and three families of F_3 progenies of the cross red noncluster × green cluster; the tests for goodness of fit for the single-factor and joint segregations for each generation and for the combined populations; and the tests for agreement between generations*

Generation	Frequencies				Degrees of freedom	$R_1^{RO}r_1^{ro}$ segregation		*Clcl* segregation		Joint segregation	
	$R_1^{RO}Cl$	$R_1^{RO}cl$	$r_1^{ro}Cl$	$r_1^{ro}cl$		χ^2	Probability	χ^2	Probability	χ^2	Probability
F_2	441	53	57	121	1	.7937	.....	.2857	.....	238.0952	.....
F_3	218	30	31	53	1	.0161	.....	.0000	.....	87.7323	.....
Total	659	83	88	174	2	.8098	.....	.2857	.....	325.8275	.....
Calculated 9 : 3 : 3 : 1	564.75	188.25	188.25	62.75	.....	.....	.....	.....	.....	.....	.....
Deviation	.....	.....	.....	.....	1	.6428	.44	.1912	.67	324.3630	.00
Heterogeneity	.....	.....	.....	.....	1	.1670	.69	.0945	.76	1.4645	.23

BACKCROSS POPULATION

The detection of linkage was applied also to the backcross progeny. The number of segregates is very small, but the reduction of the data should be of some value as a supplement to the determinations made from the F_2 and F_3 progenies. The following formulas, referred to by Mather (*13*), were used in calculating the χ^2 values for the three components:

$$\chi^2 R_1^{RO} = (R_1^{RO}Cl + R_1^{RO}cl - r_1^{ro}Cl - r_1^{ro}cl)^2/n$$

$$\chi^2 Cl = (R_1^{RO}Cl - R_1^{RO}cl + r_1^{ro}Cl - r_1^{ro}cl)^2/n$$

$$\chi^2 \text{ linkage} = (R_1^{RO}Cl - R_1^{RO}cl - r_1^{ro}Cl + r_1^{ro}cl)^2/n$$

The phenotypic-class frequencies and the test of goodness of fit for the single-factor and joint segregations are given in table 5. The deviations of the R_1^{RO} and *Cl* segregations from expectation were not significant, the probability values being 0.18 and 0.50, respectively. However, the χ^2 for the joint segregation is highly significant, and it is shown that the phenotypic frequencies do not conform to those expected for independent inheritance.

Since in both groupings of the F_2 and F_3 phenotypic frequencies and in the backcross generation the single-factor ratios account for a very small part of the total χ^2, and the large component corresponds to the joint segregation, there is undoubtedly evidence of linkage of the R_1^{RO} and *Cl* factors.

TABLE 5.—*Phenotypic-class frequencies of the backcross* ($r_1^{ro}cl/R_1^{RO}Cl \times r_1^{ro}cl/r_1^{ro}cl$) *and the test for goodness of fit for the single-factor and joint segregations*

Values observed or calculated	Frequencies				Degrees of freedom	$R_1^{RO}r_1^{ro}$ segregation		*Clcl* segregation		Joint segregation	
	$R_1^{RO}Cl$	$R_1^{RO}cl$	$r_1^{ro}Cl$	$r_1^{ro}cl$		χ^2	Probability	χ^2	Probability	χ^2	Probability
Observed	52	10	14	64	1	1.829	.18	.457	.50	60.457	.00
Calculated 1:1:1:1	35	35	35	35							

ESTIMATION OF LINKAGE VALUES

F_2 AND F_3 POPULATIONS

As pointed out by Sturtevant and Beadle (*15*), the estimation of linkage values from data of a backcross to the double-recessive parental line is more direct and more efficient than the estimation of values from F_2 or F_3 data. However, in cotton only a few seeds are obtained from each pollination, and it is difficult to obtain a large backcross population. Since the linkage under study is not very strong and since the recessive allelomorphs are both contributed by the same parent, it is thought that the calculation of the recombination percentages from the F_2 and F_3 data is not particularly inefficient.

The linkage values, based upon the recombination frequencies from the F_2 and F_3 data, were estimated by the use of the product formula described by Fisher and Balmukand (*3*) and by Immer (*9*). Product-ratio values were determined, and the corresponding recombination fractions were obtained from tables prepared by Stevens (*14*). The percentage of recombinations, as estimated from the combined F_2 and F_3 progenies of the four families, is 18.5.

THE BACKCROSS POPULATIONS

The linkage values, based upon the recombination frequencies of the backcross, were estimated from the small population of 140 segregates. Calculations resulted in a cross-over value of 17.1 percent.

HETEROGENEITY OF THE GROUPS IN RESPECT TO THE RECOMBINATION FRACTIONS

F_2 AND F_3 POPULATIONS

By assuming homogeneity, the cross-over value 0.18499, which was estimated from the total F_2 and F_3 population, may be used in the formulation of the expected segregation of the various groups. The expected frequencies of the four phenotypic classes of the F_2 and F_3 generations were determined by the following formulas:

$$R_1^{RO}Cl = \frac{n}{4}(2+P)$$

$$R_1^{RO}cl = \frac{n}{4}(1-P)$$

$$r_1^{ro}Cl = \frac{n}{4}(1-P)$$

$$r_1^{ro}cl = \frac{n}{4}P$$

where $P=(1-p)^2$ and p is the recombination fraction.

In table 6 the goodness of fit test, based upon the agreement between the observed F_2 and F_3 phenotypic frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the combined populations, is given. Following the suggestion of Mather (*13*), the two singly dominant classes of each family were added together for purposes of estimation, since they have the same expectation in terms of P. The contributions of the four families to the χ^2 are 0.0794, 1.7848, 0.3872, and 2.0031, making a total of 4.2545. There are seven degrees of freedom, since the three classes of each family contributed two degrees, one of the total of eight being lost in estimating the linkage value. The resulting probability value is 0.75, and it is concluded that the deviation of the four families from expectation is not significant.

TABLE 6.—*Goodness of fit test, based upon the agreement between the observed F_2 and F_3 phenotypic frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the combined populations* ($p=0.66424$)

Family	Frequencies		χ^2 $(O-C)^2/C$
	Observed	Calculated	
1	80	79.9272	0.0001
	21	20.1456	.0362
	19	19.9272	.0431
Total	120	120.0000	.0794
2	175	184.4986	.4890
	54	46.5028	1.2087
	48	45.9986	.0871
Total	277	277.0000	1.7848
3	122	123.2211	.0121
	34	31.0578	.2787
	29	30.7211	.0964
Total	185	185.0000	.3872
4	282	281.0773	.0030
	62	70.8454	1.1044
	78	70.0773	.8957
Total	422	422.0000	2.0031
Total χ^2			[1] 4.2545

[1] Degrees of freedom = 7; probability = 0.75.

In table 6 the F_2 and F_3 segregating progenies were combined in three of the families. Such combining of generations is not justified unless it is known that there is no significant difference between the F_2 and F_3 recombination fractions. The goodness of fit test, based upon the agreement of the observed phenotypic frequencies of the F_2 and F_3 generations and the expected frequencies, assuming the recombination fraction of the combined populations, is given in table 7. The χ^2 values for the F_2 and F_3 segregates are 0.9605 and 0.6239, respectively, making a total of 1.5844. There are three degrees of freedom, and the probability value is 0.67. The observed phenotypic frequencies of the F_2 and F_3 segregations agree satisfactorily with the assumed frequencies, based upon the cross-over value of 18.5 percent.

TABLE 7.—*Goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the combined F_2 and F_3 generations and the expected frequencies, assuming the recombination fraction of the combined populations ($p=0.66424$)*

Generation	Frequencies		χ^2 $(O-C)^2/C$
	Observed	Calculated	
F_2	441	447.5923	0.0971
	110	112.8154	.0703
	121	111.5923	.7931
Total	672	672.0000	.9605
F_3	218	221.1319	.0444
	61	55.7362	.4971
	53	55.1319	.0824
Total	332	332.0000	.6239
Total χ^2			[1] 1.5844

[1] Degrees of freedom=3; probability=0.67.

TABLE 8.—*Goodness of fit test, based upon the agreement between the observed F_2 and F_3 frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the respective family*

Family	$P=(1-p)^2$	Frequencies		χ^2 $(O-C)^2/C$
		Observed	Calculated	
1	.65012	80	79.5036	0.0031
		21	20.9928	.0000
		19	19.5036	.0130
Total		120	120.0000	.0161
2	.62500	175	181.7813	.2530
		54	51.9375	.0819
		48	43.2812	.5145
Total		277	277.0000	.8494
3	.63581	122	121.9062	.0001
		34	33.6876	.0029
		29	29.4062	.0056
Total		185	185.0000	.0086
4	.71604	282	286.5422	.0720
		62	59.9156	.0725
		78	75.5422	.0800
Total		422	422.0000	.2245

Mather (*13*) has suggested that the goodness of fit test may be made more sensitive by basing the formulation of the expected phenotypic frequencies of each group of segregates upon the respective recombination fraction of the individual group, determining the total χ^2 value, and comparing the value with the χ^2 calculated upon the basis of the joint estimate of the recombination fraction. In table 8 the goodness of fit test, based upon the agreement between the observed F_2 and F_3 frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the respective family, is given. The χ^2 values for the four families are 0.0161, 0.8494, 0.0086, and 0.2245. Subtraction of the sum of these values from the total χ^2 calculated on the basis of the recombination fraction of the combined populations (table 6), results in the χ^2

3.1559. This difference is due to discrepancies in the recombination fractions shown by the four families and has three degrees of freedom, the probability value being 0.38. The four families agree in the recombination fraction that they show.

Similarly, the more sensitive test may be applied in determining the significance of the discrepancies in the recombination fractions shown by the F_2 and F_3 segregations. The goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the individual generations and the expected frequencies, assuming the recombination fraction of the respective generation, is given in table 9. The χ^2 values for the F_2 and F_3 segregations are 0.6462 and 0.0054, respectively. Subtraction of the sum of these values from the χ^2 calculated on the basis of the recombination fraction of the combined population (table 7), results in the χ^2 value 0.9328. Since this remainder is concerned with the difference between the two segregations, there is one degree of freedom, and the probability value is 0.35. It is concluded that the two sets of data do not deviate significantly from the linkage value (18.5 percent).

TABLE 9.—*Goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the individual generation and the expected frequencies, assuming the recombination fraction of the respective generation*

Generation	$P=(1-p)^2$	Frequencies		$\frac{\chi^2}{(O-C)^2/C}$
		Observed	Calculated	
F_2	.67907	441	450.0838	0.1833
		110	107.8324	.0436
		121	114.0838	.4193
Total		672	672.0000	.6462
F_3	.63355	218	218.5847	.0016
		61	60.8306	.0005
		53	52.5847	.0033
Total		332	332.0000	.0054

BACKCROSS POPULATION

The cross-over value for the small backcross progeny ($r_1^{ro}\,cl/R_1^{RO}C1 \times r_1^{ro}cl/r_1^{ro}cl$) has been shown to be 17.143 percent. The agreement of the observed backcross phenotypic-class frequencies with the calculated frequencies, based upon the percentage of recombinations, is shown in table 10. The expectations of the four phenotypic classes were determined by the following formulas:

$$R_1^{RO}Cl=\frac{n}{2}(1-p)$$

$$R_1^{RO}cl=\frac{n}{2}(p)$$

$$r_1^{ro}Cl=\frac{n}{2}(p)$$

$$r_1^{ro}cl=\frac{n}{2}(1-p)$$

where p equals the recombination fraction. The χ^2 value is 1.908, which corresponds to a probability value of 0.40, since there are two degrees of freedom, one degree having been lost in the estimation of linkage.

TABLE 10.—*Goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the backcross* ($r_1{}^{ro}cl/R_1{}^{RO}Cl \times r_1{}^{ro}cl/r_1{}^{ro}cl$) *and the expected frequencies, assuming the recombination fraction of the backcross segregates* ($p=0.17143$)

Phenotypic class	Frequencies		x^2
	Observed	Calculated	
$R_1{}^{RO}Cl$	52	58	0.6207
$R_1{}^{RO}cl$	10	12	.3333
$r_1{}^{ro}Cl$	14	12	.3333
$r_1{}^{ro}cl$	64	58	.6207
Total	140	140	[1] 1.9080

[1] Degrees of freedom=2; probability=0.40.

The cross-over value (17.1 percent) obtained from the backcross data differs from the value (18.5 percent) obtained in the F_2 and F_3 segregation of the four families. In table 11 the goodness of fit test, based upon the agreement of the observed phenotypic frequencies of the backcross and the expected frequencies, assuming the recombination percentage 18.57 of the total population of F_2 and F_3 segregates of the four families, is given. The observed and expected frequencies agree satisfactorily, the probability value being 0.5. It is assumed that the cross-over value of the backcross population does not differ greatly from the value (18.5 percent) determined from the 1,004 F_2 and F_3 segregates of the four families.

TABLE 11.—*Goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the backcross* ($r_1{}^{ro}cl/R_1{}^{RO}Cl \times r_1{}^{ro}cl/r_1{}^{ro}cl$) *and the expected frequencies, assuming the recombination fraction of the total population of the* F_2 *and* F_3 *segregates of the four families* ($p=0.18499$)

Phenotypic class	Frequencies		x^2
	Observed	Calculated	
$R_1{}^{RO}Cl$	52	57.0507	0.4471
$R_1{}^{RO}cl$	10	12.9493	.6717
$r_1{}^{ro}Cl$	14	12.9493	.0853
$r_1{}^{ro}cl$	64	57.0507	.8465
Total	140	140.0000	[1] 2.0506

[1] Degrees of freedom=3; probability=0.57.

SUMMARY AND CONCLUSIONS

Crosses were made between two strains of upland cotton characterized respectively by red plant, noncluster habit ($R_1{}^{RO}R_1{}^{RO}Cl\ Cl$) and green plant, cluster habit ($r_1{}^{ro}\ r_1{}^{ro}\ cl\ cl$).

Although the F_1 differed slightly from the noncluster parent, the noncluster characteristic is dominant, or practically so.

In the F_2 and backcross generations, good 3 : 1 and 1 : 1 ratios of noncluster to cluster were obtained, verifying the previous conclusion that the characteristic is controlled by one genetic-factor pair.

Detailed studies in linkage detection and linkage estimation show that the $R_1{}^{RO}$ and Cl genes belong to the same linkage group and that the percentage of recombinations under the conditions of this study is not far from 18.5.

These results verify the previous reports regarding the existence of the linkage of the two genes. However, the recombination fraction determined in these studies is somewhat higher than that previously reported.

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INHERITANCE OF A LEAF VARIEGATION IN BEANS[1]

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INTRODUCTION

In the course of investigations for the development of mosaic resistance in beans (*Phaseolus vulgaris* L.), a leaf variegation was frequently observed in progenies from hybrids in which Corbett Refugee, a selection from Stringless Green Refugee, had been used as one of the parents and also in progenies from hybrids involving other parental stock. The symptoms, which somewhat resembled those caused by a virus, differed from those of the common bean mosaic, and repeated attempts to infect susceptible bean varieties by the usual methods proved that this abnormality was not of a virus nature. It has been demonstrated by a study of these hybrids that this variegation is heritable (*7*).[3]

Other heritable leaf abnormalities and chlorophyll-deficient types in beans have been described by Burkholder and Muller (*1*), Parker (*3*, *4*), Johannsen (*2*), and Tjebbes and Kooiman (*6*).

In 1936 Harrison and Burkholder,[4] and some time later Horsfall, Burkholder, and Reinking,[5] reported in Wisconsin Refugee a disease which appeared to them to be a new virus disease of beans and which they called "one-sided mosaic". The symptoms described for this disease coincide almost exactly with the abnormality described in this paper.

In 1939 Reinking and Withiam (*5*) found that in certain bean plantings in New York as many as 22 to 27 percent of variegated plants were present in Idaho Refugee and in Corbett Refugee, one of the parents of Idaho Refugee. The variegation was not identical in all respects with the condition described in this paper.

The writer has observed variegation in commercial plantings of Wisconsin Refugee and Idaho Refugee beans grown in Colorado and Idaho. Both varieties are of the same parentage. Although the percentage of affected plants was small in most cases, it was not difficult to find abnormal plants.

Two kinds of leaf variegation were encountered in these studies: (1) A type that appeared on the primary leaves and later on the trifoliate leaves if the plant survived, the symptoms on these leaves frequently being more severe than those on the simple leaves; and (2) a type with similar symptoms confined to the trifoliate leaves. This second type of variegation was most common under field conditions.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 126.

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The symptoms of these two types are impossible to differentiate on the trifoliate leaves. Plants with extremely variegated primary leaves usually die. In some cases the symptoms of the type confined to the trifoliate leaves are not noted until the plant has reached considerable size. When these studies were initiated it was assumed that such symptoms noted only on the trifoliate leaves were a delayed expression of the variegation that also appeared on the primary leaves. It has since been determined that the primary-leaf variegation is inherited independently of the type that occurs only on the trifoliate leaves and is governed by different genetic factors. These studies deal only with the inheritance of the leaf variegation that appears on the primary leaves of seedling plants. The data presented are based on results of crosses between Corbett Refugee and several other varieties of beans and of other crosses between variegated and normal green plants.

MATERIAL AND METHODS

The crosses involved in this study are listed in table 1. The hybrids tested in 1937 and 1938 were not made for the purpose of studying the inheritance of variegation; they were made for breeding disease-resistant varieties. The variegation character was not known at that time, and hence the exact nature of all the plants used in the crosses with regard to variegation was not recorded. The symptoms of variegation in Corbett Refugee are in most instances very mild and are readily overlooked. The strain of Corbett Refugee used in the crosses made in 1937 was of this kind, and the variegation was not detected. No record was made of the presence of variegation in the strains of Corbett Refugee used in the crosses tested in 1938, but it may be assumed that no plants showing any unusual abnormality would have been used in hybridization. The Corbett Refugee and the unnamed varieties of which one of the parents was Corbett Refugee, used in the crosses tested in 1939 varied; in some plants the symptoms of variegation were not observed, whereas in others variegation was present in different degrees (see table 1). When selfed, progenies from the parents contributing the factors for variegation produced only a small percentage of variegated plants (see table 2), whereas those from the green parents bred true for the normal green condition.

The crosses (see table 1) were made in the greenhouse at the United States Horticultural Station at Beltsville, Md. The F_1 hybrids were grown under field conditions at Greeley, Colo., and remained free from the common bean mosaic and other virus diseases. Although it is known that natural field hybridization may occur in beans, this was not detected from a study of other characters, and hence it in no way accounted for the deviations which are noted in the segregating generations. The seed of each plant was harvested separately and used later for a study of the F_2 generation. These progenies were grown in the greenhouse, the temperature of which was maintained at from 70° to 80° F., and the plants were classified as soon as the simple leaves were fully developed. Certain F_2 plants were chosen at random within the normal-appearing and variegated classes and grown to maturity to give rise to the F_3 families. The F_3 families were grown both under field conditions and in the greenhouse and were classified in the seedling stage to obtain the F_2 genotypic distribution.

The data were subjected to the χ^2 test for goodness of fit to certain theoretical ratios. This test was applied to F_2 families independently and to F_3 families from F_2 plants.

SYMPTOMS

The symptoms of the leaf variegation described herein always appear on the primary leaves (fig. 1, *G–I*). They may vary considerably, with some leaves practically devoid of the normal green pigmentation (fig. 1, *H*) and with only small islands of light-green tissue interspersed with yellow. In some cases the leaf is almost entirely light-yellowish green in color with small islands of darker green tissue (fig. 1, *H*), whereas in others small islands of dark green may be surrounded by deep-yellow-colored tissue. Occasionally a leaf may show over a small portion of its surface only a slight chlorotic streaking or mottling, which may be readily overlooked. Leaves that are mostly devoid of the green pigment are smaller than those with more chlorophyll, and they usually die (fig. 1, *H*). It is probable that such plants would be overlooked under field conditions unless examinations were made early in their growth.

Symptoms on the trifoliate leaves also are variable. Sectoring is common, with only small portions of the leaf yellow and the remaining portion normal green (fig. 1, *A*, *C*). Not infrequently only a few islands of yellow are noticeable. A very characteristic symptom on plants that have not died in the seedling stage is a variegation of only one side of the leaflet (fig. 1, *A*). Symptoms of this kind were pointed out by Harrison and Burkholder[6] and by Horsfall et al.[7] The unaffected portion grows normally, whereas the chlorotic half does not. Sometimes growth almost completely stops on the chlorotic side of the leaflet; thus, the leaflet becomes decidedly distorted and curls toward the affected portion (fig. 1, *A*). This peculiar symptom suggested the name "one-sided mosaic" to Horsfall et al.[7]

A necrotic streaking or spotting may occur on the petiole and affected parts of the leaflet. The trifoliate leaflets that arise from the necrotic side of the petiole may be affected and only one or sometimes two of the leaflets remain normal (fig. 1, *C*). In like manner, only a portion of the plant may be so affected.

Seriously variegated plants that do not die in the seedling stage sometimes become somewhat rosetted and considerably stunted. The internodes are much shorter than those of normal plants, and adventitious buds commonly arise in the axils of the leaves. Affected plants grown under ideal conditions may sometimes attain maturity and produce a few curled and distorted pods. Plants mildly affected may produce a normal seed crop.

The pods on extremely variegated plants are distorted, frequently curled, and abnormal in shape, size, and color (fig. 1, *D–F*). They sometimes produce seeds that, although appearing normal in most respects, are subnormal in size.

[6] See footnote 4.
[7] See footnote 5.

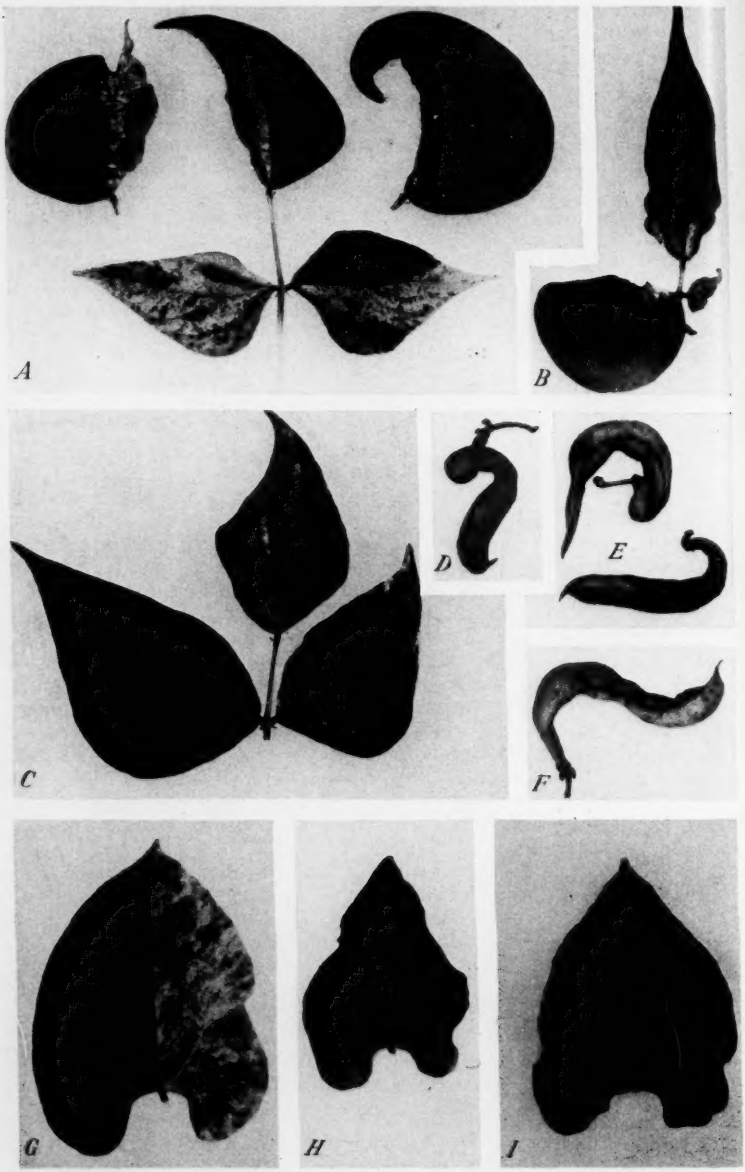

FIGURE 1.–Symptoms of variegation on bean: *A*, F_2 hybrid (Corbett Refugee × Stringless Green Refugee) showing malformation of one side of certain trifoliate leaflets; *B*, trifoliate leaf of another hybrid of same cross with extreme malformation and dwarfing of one leaflet; *C*, trifoliate leaf with only two of the three leaflets showing the variegation; *D–F*, pod malformation due to variegation; *G–I*, variegated primary leaves of Wisconsin Refugee.

EXPERIMENTAL RESULTS

PARENTS

As mentioned previously, variegation in Corbett Refugee was in most instances very mild and was readily overlooked. Although in some instances it was quite evident, it was seldom as severe as that in the segregating generations. In no case did any of the strains of Corbett Refugee or other variegated parents used in the crosses (table 1) breed true for variegation. This fact tends to explain certain results discussed later. The breeding behavior of a number of variegated parents when selfed is recorded in table 2, where it is seen that only a small percentage of their offspring manifested variegation.

TABLE 1.—*F_2 segregations from crosses between Corbett Refugee or other strains or varieties contributing the genes controlling variegation and normal green varieties*

Parentage of crosses	Nature of parent contributing variegation	Year tested	Number of F_2 plants that were—		χ^2 for 15 : 1 ratio [1]
			Green	Variegated	
U. S. No. 1 Refugee × Corbett Refugee	Normal appearing	1937	390	28	0.147
Corbett Refugee × U. S. No. 1 Refugee	do	1937	566	52	4.962
Corbett Refugee × Stringless Green Refugee	do	1937	171	4	4.658
Corbett Refugee × Perry Marrow	Unknown	1938	1,502	85	2.169
Perry Marrow × Corbett Refugee	do	1938	308	19	.108
Geneva Red Kidney × Corbett Refugee	do	1938	1,055	62	.930
Corbett Refugee × Geneva Red Kidney	do	1938	713	29	6.960
Canadian Wonder Bush × Corbett Refugee	do	1938	418	27	.025
Bountiful × Corbett Refugee	Normal appearing	1938	228	23	3.620
Small White × Corbett Refugee	Unknown	1938	332	17	1.127
Unnamed variety (9D)[2] × Blue Lake	Seriously variegated	1939–40	860	39	5.615
Unnamed variety (10A)[2] × Small White	Mildly variegated	1939–40	276	9	4.641
Wisconsin Refugee [3] × Red Kidney	Seriously variegated	1939–40	201	15	.177
Small White × Corbett Refugee	Normal appearing	1939–40	830	47	1.184
Red Kidney × Corbett Refugee	Moderately variegated	1939–40	410	29	.099
Corbett Refugee × Red Kidney	do	1939–40	116	5	.948
Perry Marrow [4] × Great Northern	Unknown	1938	226	15	.001
Cranberry [4] × Pinto	Normal appearing	1938	338	32	3.658
Unnamed variety [3] × No. 780 [5]	Variegated	1938	175	10	.245
Red Kidney [4] × Blue Lake	Normal appearing	1939–40	305	16	.892

[1] 5-percent point = 3.841. [2] Corbett Refugee, one of parents. [3] Variegated parent.
[4] Parent contributing genes controlling variegation. [5] Number carried in file of writer.

TABLE 2.—*Breeding behavior of a number of the selfed variegated parents used in the several crosses*

Variegated parent	Strain No. [1]	Number of plants that were—		
		Normal appearing	Variegated	Doubtful
Corbett Refugee	473–B	801	18	0
Do	473–5–B	781	22	25
Do	473–7–B	1,140	12	2
Do	8C	503	3	0
Do	5D	423	4	0
Wisconsin Refugee	6A	429	2	0
Unnamed variety	10A	422	10	0
Do	9D	342	2	0

[1] Numbers carried in files of writer.

F_1 GENERATION

The F_1 plants from reciprocal crosses between Corbett Refugee, as well as certain unnamed varieties, and normal green varieties were all normal green, which indicated that the factor or factors for variegation were recessive to normal green in inheritance. In some cases the Corbett Refugee parent was variegated; in others it was not. The nature of the parents used in the crosses is shown in table 1.

Reciprocal crosses were not made with every hybrid combination, but where they were made and tested they behaved about alike (table 1). This proved that the inheritance was not cytoplasmic.

F_2 GENERATION

From 20 progenies comprising 40 crosses, 9,983 F_2 plants were grown, a part of these being produced in the greenhouse and the others in the field. Fifteen of the twenty progenies showed satisfactory fits to a 15:1 ratio, as is seen in table 1. The other progenies showed significant deviations from this ratio, but only 1 was highly significant. Thirteen of the progenies had deficiencies of variegated recessives.

Although differences in the intensity of the variegation were observed, the plants were not classified as to degree of severity but were recorded only as variegated. Environment, without doubt, influenced the expression of the character, but the wide variation from a mild mottling to extreme malformation could not be reasonably accounted for on the basis of environmental factors alone, since various degrees of variegation occurred simultaneously.

F_3 GENERATION

In the F_3 generation no family of less than 66 plants was used in the analysis.

F_3 progenies were grown from 204 normal green F_2 plants that were derived from 18 original crosses. Of these, 89 progenies bred true for normal green, 61 segregated into 15 green to 1 variegated plant, and 54 segregated into 3 green to 1 variegated plant, which approximated a 7:4:4 ratio, respectively, with a χ^2 value of 1.207.[8] This substantiated the duplicate-factor hypothesis as found in the F_2 generation. Of the 61 progenies that segregated into a 15:1 ratio, 26 were deficient in variegated plants. Among the families that showed a 3:1 segregation, practically every one showed this deficiency.

DOUBLE RECESSIVE CLASS

Several hundred F_2 variegated seedlings were transplanted in order that they might be grown to maturity, but because of the lack of chlorophyll only a small percentage survived. At the beginning of the study the number of transplanted variegated seedlings was not recorded since it was not assumed that they were lethal, and as a result the exact percentage of plants that survived could not be calculated. Later studies, however, showed that from 849 transplanted seedlings derived from 136 F_3 progenies, only 119 plants or 14.0 percent survived and those plants that grew to maturity produced a relatively small number of seeds. In another experiment, 35 variegated seedlings derived from 4 F_2 progenies were transplanted. Only 11 of them survived, and these produced 105 seeds.

It is seen from table 3 that from a total of 139 variegated progenies that survived and were grown in the next generation, only 23 produced all variegated plants. There were only 83 plants produced from these

[8] 5-percent point=5.991.

23 progenies, or an average of less than 4 plants per progeny. It is probable that if the populations had been larger all progenies would have produced some green plants. Among the 37 progenies that produced all green plants, the individual populations were likewise too small to permit definite conclusions. The 79 progenies that produced 230 variegated and 427 green plants likewise had small individual populations. It is probable that if the F_2 variegated plants had produced fairly large progenies all of them would have contained both green and variegated plants.

TABLE 3.—*Breeding behavior of variegated F_2 plants in the F_3 generation*

F_2 segregation	Number of F_3 progenies	Number of F_3 plants that were—	
		Green	Variegated
All green plants	37	124	0
Green and variegated plants	79	427	230
All variegated plants	23	0	83
Total	139	551	313

Since the variegated parents used in the crosses did not breed true for variegation and since the segregating progenies in the F_2 and F_3 generations were deficient in variegated plants, it should not be expected that the double recessive lines would breed true for variegation. As mentioned previously, the progenies that produced all variegated plants were of such small populations that the data appear hardly conclusive. In general the data corroborate those of the parental material as well as those of many of the segregating progenies in that a great number showed deficiencies of variegated plants.

DISCUSSION

The results of crossing Corbett Refugee, as well as several other strains and varieties of beans that carry the factors for variegation, with normal green plants indicate that this condition is heritable and is governed by two major Mendelian factors behaving as recessives. It is evident that the data do not conform in every detail to the hypothesis of duplicate factors.

In spite of the fact that it was shown that the Corbett Refugee parents produced only a relatively few variegated offspring (table 2), it is believed that they were homozygous recessive for both variegated genes. The fact that the expression of variegation was much more pronounced in the F_2 segregates than in Corbett Refugee suggests that one or several inhibiting factors are carried by Corbett Refugee. The belief that such modifying factors exist is further strengthened by the deficiency of recessives in the F_2 generation and in most of the F_3 segregating families and also by the failure of many of the variegated recessives to breed true.

As mentioned earlier, a high percentage of the variegated plants were lethal, and it is assumed that these are the true-breeding recessives. Those that survived and produced seed were probably the ones carrying some of the inhibiting factors, possibly in the heterozygous condition. In the progeny of these plants the variegated

character may again have been suppressed, the action of the inhibitors accounting for the high percentage of green plants in the progeny of the recessive class.

This heritable leaf variegation has not been observed in many bean varieties that are grown commercially, but it has been of importance in the Wisconsin Refugee variety and to a lesser degree in the Idaho Refugee. Both of these varieties have Corbett Refugee as one of the parents. This variety has been used extensively in hybridization because of its immunity to the common bean mosaic virus. In practically all cases where it was used, the progenies exhibited variegation. Although there may be strains of Corbett Refugee that are free from this condition, it should be used as a parent only with caution. This weakness should be recognized and watched for carefully. Since inhibiting factors may suppress the expression of the variegated symptoms, normal-appearing strains may actually carry the factors for this condition and when outcrossed with normal green varieties the variegation would be expressed. If a mosaic-resistant Refugee type bean is desired as a parent, it is suggested that the U. S. No. 5 Refugee had better be used. This variety, although derived from a cross with Corbett Refugee, is free from variegation and is resistant to the common bean mosaic.

SUMMARY

Two types of leaf variegation have been observed in beans, one of which appears both on the primary and the trifoliate leaves and the other only on the trifoliate leaves. The symptoms of the two are similar, but they are inherited differently. The inheritance of the first is described in this paper.

The results obtained when plants of Corbett Refugee and other varieties that carry the factors for variegation were crossed with normal green plants of several varieties are presented. For the most part the data support the two-factor Mendelian hypothesis. The F_1 plants of reciprocal crosses were normal green in appearance, and a ratio of 15 green plants to 1 variegated was obtained in the F_2 generation.

In the F_3 generation from green F_2 plants, a 7:4:4 ratio of all green, 15 green to 1 variegated, and 3 green to 1 variegated was obtained.

The variegated recessive progenies, except in a few instances where the populations were small, did not breed true in the F_3 generation. It is assumed that this lack of true breeding was due to one or several inhibiting factors that suppressed the variegation character. The death of a high percentage of the variegated plants was probably due to the absence of these inhibitors.

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